PB83-133066

Selected Aliphatic Amines and Related Compounds: An Assessment of the Biological and Environmental Effects

(U.S.) National Research Council
Washington, DC

Prepared for

Environmental Protection Agency
Washington, DC

1981

U.S. Department of Commerce
National Technical Information Service
NTIS

NRC NAS NAE IOM

PB83-133066

Selected Aliphatic Amines and Related Compounds: An Assessment of the Biological and Environmental Effects

Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences
National Research Council

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

50272-101

REPORT DOCUMENTATION PAGE

1. REPORT NO.

2.

3. Recipient's Accession No. PB83 133066

4. Title and Subtitle

Selected aliphatic amines and related compounds: an assessment of the biological and environmental effects

5. Report Date 1981

6.

7. Author(s) Committee on Amines

8. Performing Organization Rept. No.

9. Performing Organization Name and Address

National Research Council
Board on Toxicology and Environmental Health Hazards
2101 Constitution Ave NW
Washington, D.C. 20418

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.

(C) 68-01-4655

(G)

12. Sponsoring Organization Name and Address

U.S. Environmental Protection Agency

13. Type of Report & Period Covered Final

14.

15. Supplementary Notes

See also Aromatic amines: an assessment of the biological and environmental effects

16. Abstract (Limit: 200 words)

The greatest potential of aliphatic amines for the induction of acute and long-term toxic effects arises from their ability to be nitrosated to nitrosamines that are genotoxic with potential for mutagenicity, teratogenicity, and carcinogenicity, among other toxic effects. The report contains chapters on triethanolamine, morpholine, and 2-nitropropane. Triethanolamine is used in industry and in cosmetic preparations. It is also converted by nitrosating agents to N-nitrosodiethanolamine, which has been known for some time to induce cancer in rodents. Morpholine is an industrially important secondary amine that is nitrosated to N-nitrosomorpholine, which is carcinogenic in animals. 2-Nitropropane was selected because of its dispersion into the environment resulting from its use in paints and other coatings.

The following recommendations were made:

1) The potential for 2-nitropropane, or the nitrite liberated from it, to form N-nitroso compounds in vivo urgently needs attention.

2) There is a need to consolidate present understanding of the carcinogenicity of 2-nitroprropane obtained through experimentation by studying its effect in species other than the rate and by determining whether it is effective at doses of 25 ppm or lower.

3) The epidemiologic study of workers producing 2-nitropropane should be continued to ensure that a sufficient number of exposed persons have survived long enough to

17. Document Analysis a. Descriptors

Mitrosation; Toxic substances

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement:

This report has been approved for public sale; its distribution is unlimited

19. Security Class (This Report) Unclassified

20. Security Class (This Page) Unclassified

21. No. of Pages 177

22. Price

(See ANSI–Z39.18) See Instructions on Reverse

OPTIONAL FORM 272 (4–77)
(Formerly NTIS–35)
Department of Commerce

DO NOT PRINT THESE INSTRUCTIONS AS A PAGE IN A REPORT

INSTRUCTIONS

Optional Form 272, Report Documentation Page is based on Guidelines for Format and Production of Scientific and Technical Reports, ANSI Z39.18–1974 available from American National Standards Institute, 1430 Broadway, New York, New York 10018. Each separately bound report—for example, each volume in a multivolume set—shall have its unique Report Documentation Page.

1. Report Number. Each individually bound report shall carry a unique alphanumeric designation assigned by the performing organization or provided by the sponsoring organization in accordance with American National Standard ANSI Z39.23–1974, Technical Report Number (STRN). For registration of report code, contact NTIS Report Number Clearinghouse, Springfield, VA 22161. Use uppercase letters, Arabic numerals, slashes, and hyphens only, as in the following examples: FASEB/NS–75/87 and FAA/RD–75/09.
2. Leave blank.
3. Recipient's Accession Number. Reserved for use by each report recipient.
4. Title and Subtitle. Title should indicate clearly and briefly the subject coverage of the report, subordinate subtitle to the main title. When a report is prepared in more than one volume, repeat the primary title, add volume number and include subtitle for the specific volume.
5. Report Date. Each report shall carry a date indicating at least month and year. Indicate the basis on which it was selected (e.g., date of issue, date of approval, date of preparation, date published).
6. Sponsoring Agency Code. Leave blank.
7. Author(s). Give name(s) in conventional order (e.g., John R. Doe, or J. Robert Doe). List author's affiliation if it differs from the performing organization.
8. Performing Organization Report Number. Insert if performing organization wishes to assign this number.
9. Performing Organization Name and Mailing Address. Give name, street, city, state, and ZIP code. List no more than two levels of an organizational hierarchy. Display the name of the organization exactly as it should appear in Government indexes such as Government Reports Announcements & Index (GRA & I).
10. Project/Task/Work Unit Number. Use the project, task and work unit numbers under which the report was prepared.
11. Contract/Grant Number. Insert contract or grant number under which report was prepared.
12. Sponsoring Agency Name and Mailing Address. Include ZIP code. Cite main sponsors.
13. Type of Report and Period Covered. State interim, final, etc., and, if applicable, inclusive dates.
14. Performing Organization Code. Leave blank.
15. Supplementary Notes. Enter information not included elsewhere but useful, such as: Prepared in cooperation with . . . Translation of . . . Presented at conference of . . . To be published in . . . When a report is revised, include a statement whether the new report supersedes or supplements the older report.
16. Abstract. Include a brief (200 words or less) factual summary of the most significant information contained in the report. If the report contains a significant bibliography or literature survey, mention it here.
17. Document Analysis. (a). Descriptors. Select from the Thesaurus of Engineering and Scientific Terms the proper authorized terms that identify the major concept of the research and are sufficiently specific and precise to be used as index entries for cataloging.

    (b). Identifiers and Open-Ended Terms. Use identifiers for project names, code names, equipment designators, etc. Use open-ended terms written in descriptor form for those subjects for which no descriptor exists.

    (c). COSATI Field/Group. Field and Group assignments are to be taken from the 1964 COSATI Subject Category List. Since the majority of documents are multidisciplinary in nature, the primary Field/Group assignment(s) will be the specific discipline, area of human endeavor, or type of physical object. The application(s) will be cross-referenced with secondary Field/Group assignments that will follow the primary posting(s).
18. Distribution Statement. Denote public releasability, for example "Release unlimited", or limitation for reasons other than security. Cite any availability to the public, with address, order number and price, if known.

19. & 20. Security Classification. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED).

21. Number of pages. Insert the total number of pages, including introductory pages, but excluding distribution list, if any.
22. Price. Enter price in paper copy (PC) and/or microfiche (MF) if known.

*U.S. Government Printing Office: 1978—261-647/3308

develop all tumors that may be induced by this chemical.

4) The chronic toxicity studies conducted for the sole manufacturer of 2-nitropropane should be peer reviewed and published.

Selected Aliphatic Amines and Related Compounds: An Assessment of the Biological and Environmental Effects

Committee on Amines
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences
National Research Council

National Academy Press
Washington, D.C.
1981

i·b

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The National Research Council was established by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and of advising the federal government. The Council operates in accordance with general policies determined by the Academy under the authority of its congressional charter of 1863, which establishes the Academy as a private, non-profit, self-governing membership corporation. The Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in the conduct of their services to the government, the public, and the scientific and engineering communities. It is administered jointly by both Academies and the Institute of Medicine. The Academy of Engineering and the Institute of Medicine were established in 1964 and 1970, respectively, under the charter of the National Academy of Sciences.

At the request of and funded by the
U.S. Environmental Protection Agency,
Contract No. 68-01-4655

List of Participants

COMMITTEE ON AMINES

DAVID B. CLAYSON, University of Nebraska Medical Center, Omaha, Nebraska, Chairman
GEORGE T. BRYAN, University of Wisconsin, Center for Health Sciences, Madison, Wisconsin
DAVID H. FINE, New England Institute for Life Sciences, Waltham, Massachusetts
CHARLES C. IRVING, Veterens Administration, Center for Health Sciences, Memphis, Tennessee
CHARLES M. KING, Michigan Cancer Foundation, Detroit, Michigan
RICHARD MONSON, Harvard School of Public Health, Boston, Massachusetts
JACK L. RADOMSKI, University of Miami, Miami, Florida
DONALD H. STEDMAN, University of Michigan, Ann Arbor, Michigan
STEVEN R. TANNENBAUM, Massachusetts Institute of Technology, Cambridge, Massachusetts
SNORRI S. THORGEIRSSON, National Cancer Institute, Bethesda, Maryland
JOHN H. WEISBURGER, Naylor Dana Institute, Valhalla, New York
ERROL ZEIGER, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Consultants

EMERICH FIALA, Naylor Dana Institute, Valhalla, New York
MALCOLM C. BOWMAN, National Center for Toxicological Research, Jefferson, Arkansas

National Research Council Staff

ROBERT J. GOLDEN, Project Director
FRANCES M. PETER, Editor
AGNES E. GASKIN, Secretary

EPA Project Officer

ALAN CARLIN, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

BOARD ON TOXICOLOGY AND ENVIRONMENTAL HEALTH HAZARDS

RONALD W. ESTABROOK, University of Texas Medical School (Southwestern), Dallas, Texas, Chairman
THEODORE CAIRNS, DuPont Chemical Co. (retired), Greenville, Delaware
VICTOR COHN, George Washington University Medical Center, Washington, D.C.
JOHN W. DRAKE, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
ALBERT M. FREEMAN, Bowdoin College, Brunswick, Maine
RICHARD HALL, McCormick & Company, Hunt Valley, Maryland
RONALD W. HART, National Center for Toxicological Research, Jefferson, Arkansas
PHILIP LANDRIGAN, National Institute for Occupational Safety and Health, Cincinnati, Ohio
MICHAEL LIEBERMAN, Washington University School of Medicine, St. Louis, Missouri
BRIAN MacMAHON, Harvard School of Public Health, Boston, Massachusetts
RICHARD MERRILL, University of Virginia, Charlottesville, Virginia
ROBERT A. NEAL, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina
IAN NISBET, Massachusetts Audubon Society, Lincoln, Massachusetts
CHARLES R. SCHUSTER, JR., University of Chicago, Chicago, Illinois
GERALD WOGAN, Massachusetts Institute of Technology, Cambridge, Massachusetts
ROBERT G. TARDIFF, National Research Council, Washington, D.C., Executive Director

CONTENTS

PAGE

Executive Summary.. 1

CHAPTER 1 - Nitrosation of Amines and Their Control........... 21

CHAPTER 2 - General Analytic Methods........................... 45

CHAPTER 3 - Epidemiology....................................... 57

CHAPTER 4 - Triethanolamine.................................... 67

CHAPTER 5 - Morpholine... 92

CHAPTER 6 - 2-Nitropropane..................................... 143

EXECUTIVE SUMMARY

In 1977, the U.S. Environmental Protection Agency (EPA) contracted with the National Academy of Sciences to prepare a series of reports on pollutants that were believed to be associated with deleterious effects in humans and the environment. As part of this effort, the agency asked the Academy to assess the health and environmental effects of selected aromatic and aliphatic amines. In response, the Committee on Amines was established within the Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences, National Research Council.

The Committee on Amines decided that the very different properties of the two classes of amines would necessitate division of the study into two separate components: one devoted to aromatic amines and related compounds and another to aliphatic amines and nitro compounds. This resulted in the preparation of two separate reports. The task was extended to related chemicals in order to include those compounds resulting from the metabolic conversion of the amines, especially those of the aromatic group. Such compounds may be formed from nitro or azo compounds and in turn give rise to hydroxylamine derivatives.

The greatest potential of ~~these chemicals~~ aliphatic amines for the induction of acute and long-term toxic effects arises from their ability to be nitrosated to nitrosamines that are genotoxic with potential for mutagenicity, teratogenicity, and carcinogenicity, among other toxic effects. Risk assessments for nitrosamines have not been attempted since the rate of nitrosation depends on the nature and concentration of the nitrosating agent and the presence of catalysts in the environment. The problem is further complicated by the fact that there are large differences in carcinogenic potency among the nitrosamines.

Because aliphatic amines generally have a low level of toxicity their odor and potential for the induction of temporary respiratory tract irritation serve as warnings against exposure. Furthermore, there is virtually no dose-response information on the related potentially carcinogenic nitrosamines. Such data could be used to estimate risk with current methodology. Consequently, risk assessment has not been attempted for these agents.

The committee was unable to adequately address the environmental aspects of exposure after an intensive search of the literature revealed a general lack of information on this subject for the compounds selected.

The report contains introductory chapters providing an overview of some general information on the amines under discussion followed by more in-depth considerations of specific substances. This report contains chapters on triethanolamine, morpholine, and 2-nitropropane. Triethanolamine is used in industry and in cosmetic preparations. It is also converted by nitrosating agents to N-nitrosodiethanolamine, which has been known for some time to induce cancer in rodents. Morpholine is an industrially important secondary amine that is nitrosated to N-nitrosomorpholine, which is carcinogenic in animals. 2-Nitropropane was selected because of its dispersion into the environment resulting from its use in paints and other coatings.

NITROSATION OF AMINES AND THEIR CONTROL

N-Nitroso compounds are formed by the interaction of nitrosating agents with a variety of amines or amides. Primary, secondary, or tertiary amines may be nitrosated to nitrosamines under acidic, neutral, or alkaline conditions. Nitrosation may be brought about by nitrous acid derived from nitrite ion, the oxides of nitrogen, nitro compounds, or by transnitrosation. Nitrosamines may be detected wherever aliphatic amines are present.

The oxides of nitrogen --dinitrogen tetraoxide (N_2O_4), dinitrogen trioxide (N_2O_3), and nitrogen dioxide (NO_2)--nitrosate much more rapidly than nitrous acid and lead to nitrosamines, to deaminated products, and, sometimes, to nitramines, especially in the presence of catalysts such as iodine, iodides, or metal salts.

The human environment is contaminated by such nitrosamines. The highest exposures have been reported for tannery workers, especially those concerned with wet tanning, for whom exposures ranging from 23 to 47 $\mu g/m^3$ have been observed. Nitrosomorpholine has been measured at levels of 0.5 to 27 $\mu g/m^3$, leading to human exposures between 50 to 250 μg daily. In a rocket fuel factory, dimethylnitrosamine, an intermediate in the manufacture of 1,1-dimethylhydrazine, resulted in daily exposure of workers to levels between 10 and 50 μg. Nitrosamines are also a component of tobacco smoke. A person who smokes 20 cigarettes per day inhales several nitrosamines in an amount totalling approximately 16.8 μg daily.

There have been extensive studies of the ingestion of nitrosamines in food, especially in preserved meat and fish products, and the formation of these compounds from the interaction of nitrites and amines in the stomach. Until recently, the maximum exposure from this source has been attributed to beer. The consumption of three cans of one brand of beer has been reported to result in the ingestion of approximately 8.4 μg of dimethylnitrosamine. The nitrosamine levels in beer have now been reduced due to changes in the manufacturing process

Average human exposure from all known sources is estimated to be approximately 280 ng of mixed nitrosamines per kilogram of body weight daily. Cigarette smoking is by far the largest single component of daily exposure totalling 240 ng/kg/day.

The formation of nitrosamines, especially in industrial products, has been successfully controlled, once their potential health effects were identified. For example, the amount of nitrosodipropylamine in the pesticide trifluralin, which has been measured at levels as high as 195,000 ppb (0.195%), was reduced by changes in the manufacturing process. The nitrosodimethylamine impurity in beer arose during drying of the moist malt. Reductions in this compound have been achieved by instituting the use of gas burners with lower nitrogen oxide content and by increasing the acidity of the malt. Other problems have been solved by similar manipulation of production conditions.

The formation of nitrosamines in bacon and other meat or fish products preserved by the addition of nitrites is inhibited by adding ascorbic acid or other nitrite sinks to the food to reduce the level of nitrite available for nitrosation.

Recommendation

The most effective way to control potentially carcinogenic nitrosamines would be the widespread dissemination of information on nitrosamine formation. Manufacturers and users of amines and nitrosating agents should be made aware that certain processes and products probably result in the formation of nitrosamines, but that the extent of contamination can be minimized by the use of appropriate technology.

GENERAL ANALYTIC METHODS

Aliphatic amines are generally analyzed by gas, paper, or thin-layer chromatography. Recent studies have used gas chromatography following derivatization, which among other advantages prevents peak tailing or "ghost" effects due to interactions of the basic amine with active sites in the column. The selection of derivative is determined by the type of gas chromatographic detector used and whether the purpose of the study is to separate primary, secondary, or tertiary amines. For example, picogram quantities of amino compounds have been measured using heptafluorobutyryl derivatives and electron capture detection. Similar principles apply for thin-layer chromatography, but the methodology is less sensitive. Thus, for example, detection of microgram amounts is possible using colored 4'-nitroazobenzyl-4-amide derivatives.

EPIDEMIOLOGIC STUDIES

Since there have been only infrequent reports of adverse reactions to aliphatic amines used in industry, few epidemiologic studies have been conducted. In contrast, nitroparaffins such as 2-nitropropane, which exhibit greater toxicity, have been examined in greater depth, but the results of even this study remains equivocal.

Certain aliphatic amines such as hexamethyleneamine or triethanolamine are apparently related to dermatologic conditions. Caution in accepting these associations is necessary since the individual worker is exposed to a wide variety of agents rather than to a single amine.

Although there is no direct evidence that the nitrosamine products of the aliphatic amines induce cancer in humans, their widespread, though low level, occurrence in the environment combined with their carcinogenicity in a wide spectrum of laboratory animal species, suggests that they may also be carcinogenic in humans.

There are two situations in which nitrosamine-induced human cancer is suspected, although not established. In Cali, Colombia, drinking water contains high levels of nitrate, which is reduced by bacteria to nitrite in the body, thereby becoming a potential nitrosating agent. These elevated high nitrate levels are associated with a high rate of human gastric cancer. In a region of China where there is a high esophageal cancer rate, elevated levels of nitrite and specific nitrosamines are present in the food supply.

Recommendations

The effects of exposure to nitrate, nitrite, and nitrosamine on the incidence of human cancer urgently need intensive epidemiologic study. Investigators conducting such studies should be equipped with a knowledge of possible concurrent exposure to other potential carcinogens, the dietary regimen and smoking habits of exposed and control populations, and the concentrations of each nitroso compounds to which the study populations are or have been exposed.

TRIETHANOLAMINE

Triethanolamine (2,2',2"-trihydroxytriethylamine) is a relatively nonvolatile substance (vapor pressure less than 0.01 mm Hg at 20°C) that boils at 335.4°C and melts at 21.1°C. It is produced in large volumes. In 1978, 52,000 mt were manufactured at several sites in the United States by the reaction of ethylene oxide and aqueous ammonia at 50 to 100°C. The mixture of mono-, di-, and triethanolamine is separated by distillation.

This chemical has a wide range of uses in soaps and detergents, textile specialities, cosmetics, agricultural products, and many other products. The figures are far greater for the use of this compound than those reported for yearly production (122,000-146,000 mt) in the United States. Part of this difference may result from doubts about its use in gas purification, and the remainder may be imported.

There is no information about the release of triethanolamine into the environment during production or conversion to other products. Food and cosmetic uses present the greatest potential for human exposure. Since triethanolamine may be nitrosated to *N*-nitrosodiethanolamine, the toxicity of both compounds must be considered.

Triethanolamine in complex mixtures can be analyzed by extracting organic matter with methylene chloride and subsequent evaporation to dryness on a steam bath. Acidic and alkaline contaminants may also be removed. The residues may be analyzed directly by high performance liquid chromatography or, after alkylation or esterification to give volatile derivatives, by gas chromatography.

Few metabolic studies of either triethanolamine or its nitrosation product have been initiated. Most of the ingested N-nitrosodiethanolamine is excreted unchanged in the urine by rats, the proportional excretion level being independent of dose over the range of 10 to 1,000 mg/kg. This nitrosamine has also been shown to be absorbed through human and shaved rat skin. This observation is important in view of the fact that the exposure of humans to cosmetic preparations containing triethanolamine, and in some cases its nitrosation product, is dermal.

The acute toxicity of triethanolamine was studied in aquatic protozoa and invertebrates. Its toxicity was less than that of diethanolamine or monoethanolamine. Acute effects were observed to result from exposures greater than 100 mg/liter, whereas chronic effects occurred above 1 mg/liter.

In mammals, all three ethanolamines are weakly toxic; no LD_{50}'s were observed at levels less than 700 mg/kg for any species or route of administration. Russian studies indicate that 13% triethanolamine solutions penetrated rat skin and led to changes in liver and the central nervous system. Topically applied triethanolamine in mice was not carcinogenic or cocarcinogenic, although it inhibited the cocarcinogenic properties of one detergent with which it was applied.

In humans, triethanolamine acts as a sensitizer to contact allergens. Workers handling cutting fluids and other mixtures containing triethanolamine, may develop dermatoses as a result of this exposure.

The nitrosation product, *N*-nitrosodiethanolamine, has been tested at high levels in rats and hamsters. Rats given 600-1,000 mg/day in drinking water (total dose, 150-300 g/kg) all developed hepatocellular carcinomas between 242 and 325 days after treatment started. Hamsters receiving *N*-nitrosodiethanolamine by subcutaneous injection on two schedules experienced a high incidence of nasal cavity tumors, papillary tumors of the trachea, and hepatocellular adenomas. These tumors were not observed in untreated control hamsters. The high doses used in rats and hamsters give no indication of the possible responses to lower doses. Further information is required in this area.

There appear to be no evidence for mutagenic activity of triethanolamine in *Salmonella* or in the *Allium cepa* mitosis test. Triethanolamine in the presence of nitrite was, however, mutagenic to _Bacillus subtilis_ in the absence, but not in the presence of rat liver S-9 fraction. Nitrosodiethanolamine is mutagenic to _Salmonella typhimurium_ and _Escherichia coli_. A metabolizing system was shown to be required by some, but not all test systems.

Recommendations

1. Suspicion that triethanolamine of unknown purity might be carcinogenic is suggested by one study. There is a need to repeat this study using a pure sample of triethanolamine under strictly defined conditions.
2. _N_-nitrosodiethanolamine, the nitrosation product of triethanolamine, is definitely carcinogenic in rats and hamsters at high exposure levels. There is a need for additional information at lower dose levels in another species to confirm these observations.
3. Mutagenicity data on _N_-nitrosodiethanolamine are inadequate and, to some extent, discordant. There is a need for further studies to determine the best microbial strains to be used for this purpose and whether a metabolizing fraction should be present. Studies with mammalian cells should also be conducted.
4. There is an absence of published data on the release of triethanolamine into the environment during production or during its many uses. This subject and its environmental persistence and fate should be investigated, especially since triethanolamine is readily nitrosated.

MORPHOLINE

Morpholine (tetrahydro-1,4-oxazine) is a colorless, hygroscopic liquid (mp -4.9°C, bp 128.9°C) with an appreciable vapor pressure (8.0 mm Hg at 20°C). It is completely miscible in water. It is produced by reacting diethyleneglycol, ammonia, and a small amount of hydrogen over a hydrogenation catalyst at 150-400°C and 30-400 atm.

Approximately 11,000 mt of morpholine is produced annually in the United States. The largest single use of the compound is in the manufacture of rubber. It is also used as a corrosion inhibitor, and the products of its interaction with fatty acids are used to form household soaps or waxes and polishes for motor vehicles. Some morpholine derivatives are present in pharmaceuticals.

Estimates of morpholine emissions into the environment do not appear to be supported by direct analytic data. It has been assumed that all morpholine used as a corrosion inhibitor (2,700 mt/yr) and in waxes or polishes (1,000 mt/year) will be emitted into the environment. This may be an overestimate. There is a need for analytically based determinations of morpholine release and persistence in the environment, especially in view of the ready nitrosation of this substance.

Morpholine may be analyzed by gas chromatography either directly or after derivatization with p-toluenesulfonyl choloride. Colorimetric analysis is possible using the dialkyldithiocarbamate complex that may be extracted into chloroform. Air monitoring of morpholine has been achieved by trapping the aliphatic amine and nitrosating it with nitrous acid to form nitrosomorpholine.

Morpholine itself has low toxicity except for its irritant effects but it is converted through nitrosation to N-nitrosomorpholine, a potent carcinogen in animals. The toxicity of morpholine and its nitroso derivative is reviewed in this report. The LC_{50} of morpholine is 2,250 ppm for male and female rats and 1,450 ppm for male and 1,900 ppm for female mice following inhalation. High levels of inhalation (12,000-18,000 ppm) for 8 h led to eye irritation, hemorrhage of the lungs, and congestion of the liver and kidneys.

In humans, 1 to 1.5 minutes of exposure to 12,000 ppm morpholine led to irritation of the nose and to coughing. Exposures of 50 to 100 ppm N-ethylmorpholine for 2 to 3 minutes led to upper respiratory tract irritation. Industrial exposure to morpholine at unspecified levels led to nasal and bronchial irritation in workers. There are divergent views on the lowest toxic level of morpholine. One author suggests that the only effect of an atmospheric concentration of 25 ppm morpholine is the ammonia-like odor; another reports that the irritant effects were observed within 1 minute at 16 ppm.

Russian studies in rats indicated that morpholine may be more toxic than is indicated in the above-mentioned reports. For example, an enhancement of thyroid activity measured by ^{131}I uptake was observed. When the rats were exposed to morpholine at 70 or 8 mg/m^3 for 4 h/day, 5 days/week for 4 months, destruction of the lymphoid structure of the spleen was recorded. Undiluted morpholine applied to the skin of rabbits and guinea pigs for 1 to 13 days proved fatal. Affected tissues included liver, spleen, and kidney. Neutralizedsolutions (morpholine salts) were non-toxic. In assessing the significance of these results, it would be helpful to know the relative purity of the morpholine samples used. However, the need for further work on the acute and sub-chronic toxicity of morpholine in animals is clearly indicated.

Chronic studies of morpholine toxicity have been conducted mainly as a control in investigations of the carcinogenicity resulting from the coadministration of morpholine and nitrite or nitric oxide or from the nitrosation product, *N*-nitrosomorpholine. In one study, morpholine alone or in the presence of low levels of nitrite slightly, but not significantly, enhanced the incidence of liver tumors in rats. This is most probably due to *in vivo* nitrosation by endogenous or added nitrite. It is unlikely to be a direct effect of morpholine.

Morpholine in the presence of substantial amounts of nitrite leads to formation of liver tumors in rats and hamsters and to lung tumors in mice. Investigators studying rats and hamsters followed the two-generation protocol in which the chemicals were fed to the mother during pregnancy and then to the offspring after parturition.

Cofeeding sodium ascorbate (Vitamin C) with morpholine and sodium nitrite inhibits the induction of tumors. The ascorbate competes with the amine for the available nitrite, thereby inhibiting the formation of _N_-nitrosomorpholine. Inhibition of tumor formation may be indicated by either a reduced number of tumors or a lengthened time to tumor occurrence.

Of particular importance to the human environment is the demonstration that ingestion of morpholine combined with the inhalation of nitric oxide has been shown to lead to _N_-nitrosomorpholine formation _in vivo_ and to enhance the incidence of lung tumors in mice. These observations should be confirmed and studies designed to demonstrate their applicability to the levels in the human environment.

Morpholine is not mutagenic in the absence of nitrosating agents. This has been established in studies using _Salmonella typhimurium_ tester strains, by chromosome studies in micronucleus tests, by mammalian embryo cell resistance to azaguanine or ouabain, or in cell transformation assays. In contrast, *N*-nitrosomorpholine was mutagenic in all of these tests and in several others.

Recommendations

1. Analytical surveys should be conducted to determine the amount of morpholine that escapes into the environment from different sources. Such information is necessary if exposure to adventitiously formed *N*-nitrosomorpholine is to be avoided.
2. The acute and sub-chronic toxicity of morpholine should be reinvestigated using defined, pure samples of the test chemical to ensure that this toxicity is due to morpholine itself and not to an occasional impurity.
3. Further studies should be conducted on the production of *N*-nitrosomorpholine by the action of atmospheric nitric oxide on ingested morpholine. Test concentrations of both compounds should approximate those to which humans may be exposed.

2-NITROPROPANE

2-Nitropropane is a moderately volatile liquid (vapor pressure: 13 mm Hg at 20°C) that is manufactured by only one U.S. producer. It is produced by reacting propane and nitric acid at 370-450°C and is separated from the accompanying

1-nitropropane, nitroethane, and nitromethane by fractional distillation. Since 2-nitropropane is used widely as a solvent in paints and other coatings, it may be expected to have a wide environmental distribution.

Emissions from the manufacture of 2-nitropropane have not been reported, but since 3,500 metric tons (mt) are used as a solvent in paints and coatings, much of this material may be released into the atmosphere. Exposure of individuals is probably greatest in industrial/commercial settings such as those involving painting, printing, and ship repairing. Traces exist in some food packaging materials. Overall, the National Institute for Occupational Safety and Health (NIOSH) estimates that 100,000 workers may be exposed to 2-nitropropane.

Metabolism in rats results in some 2-nitropropane being excreted unchanged and some being converted to acetone and nitrite or nitrate. Subsequent methemoglobinemia, which probably results from the interaction of nitrite and hemoglobin, has been observed in acute and, to a lesser extent, chronic exposure studies.

Most of the adverse health effects of 2-nitropropane have been studied after inhalation, the route of most human exposure. There is a considerable species variation in the lowest fatal level of 2-nitropropane after a 4.5-h exposure: cat, 714 ppm; rat, 1,510 ppm, rabbit, 2,380 ppm; and guinea pig, approximately 4,620 ppm. Rats, guinea pigs, rabbits, and a single monkey tolerated repeated exposure to 325 ppm or 83 ppm. Higher concentrations led to dyspnea, cyanosis, prostration, convulsions, and death.

Most chronic toxicity studies have used rats and, to a lesser extent, rabbits. In one study in rats, exposure to 400 ppm 2-nitropropane for 7 h/day led to the death of many animalsby the third day. A lower dose, 207 ppm, 7 h/day, 5 days/wk, induced either hepatocellular adenoma or carcinoma in all 10 rats sacrificed after 6 months exposure. Rats exposed for 3 months exhibited only hepatocellular hypertrophy, hyperplasia, and necrosis. In another study, no neoplasms were observed in Sprague-Dawley rats that were sacrificed after 6 months of exposure.

In a study designed to resolve the differences in response to the chronic inhalation of 2-nitropropane, Sprague-Dawley rats were exposed to 200, 100, and 25 ppm of 2-nitropropane 7-h/day, 5 days/wk for 18 months. Male, but not female, rats exhibited an increase in liver nodules compared to untreated controls. Although there is no peer reviewed, published information concerning the pathology for this series of experiments, a Health Hazard Alert published by NIOSH/OSHA (Occupational Safety and Health Administration) in October 1980 concludes that 2-nitropropane induced liver cancer in rats dosed at 200 and 100 ppm but not at 25 ppm.

In humans, four fatalities and one near fatality resulting from exposure to high doses 2-nitropropane have been recorded. The levels of exposure in these cases are unknown. The deaths occurred 6 to 10 days after exposure. Post-mortem examination revealed fatty degeneration of the liver in one case and liver necrosis in the three other cases. Lower levels of exposure to 2-nitropropane (20-45 ppm) to lead to a variety of nonspecific symptoms, especially of the digestive tract.

An epidemiologic study of the entire 1,815-person workforce of a plant manufacturing 2-nitropropane failed to associate any cause of death with exposure to 2-nitropropane. This conclusion should be viewed cautiously since only 180 deaths were recorded and only 22 years had elapsed since 2-nitropropane production was initiated. A longer study is clearly needed to ensure that 2-nitropropane is not carcinogenic in humans.

In addition to the carcinogenicity of 2-nitropropane in the rat, mutagenicity tests suggest that this chemical is also mutagenic. Positive microbial tests involving Salmonella typhimurium and Saccharomyces cerevisiae and mammalian micronucleus tests have been reported.

The Health Hazard Alert published by OSHA suggests that 2-nitropropane should be handled in the workplace as a potential carcinogen in humans. OSHA's present Permissible Exposure Limit for that compound is 25 ppm or 90 mg/m^3.

over an 8-h day on a time-weighted average. Because of the evidence for carcinogenicity in animals, it recommends that occupational exposures should be reduced to the lowest possible levels. Ways to achieve this goal are detailed in the OSHA publication.

Recommendations

1. The potential for 2-nitropropane, or the nitrite liberated from it, to form N-nitroso compounds *in vivo* urgently needs attention.
2. There is a need to consolidate present understanding of the carcinogenicity of 2-nitropropane obtained through experimentation by studying its effect in species other than the rat and by determining whether it is effective at doses of 25 ppm or lower.
3. The epidemiologic study of workers producing 2-nitropropane should be continued to ensure that a sufficient number of exposed persons have survived long enough to develop all tumors that may be induced by this chemical.
4. The chronic toxicity studies conducted for the sole manufacturer of 2-nitropropane should be peer reviewed and published

Chapter 1

NITROSATION OF AMINES AND THEIR CONTROL

Although there is concern about the adverse health effects caused by exposure to aliphatic amines, their greatest potential for damage derives from their respective nitrosamines. As noted in later chapters, the pure amines have strong tendencies to form these more toxic substances both *in vivo* and *in vitro*, as well as in the environment and there is considerable uncertainty concerning the carcinogenicity and mutagenicity of these derivatives.

Consequently, in addition to the general occurrence and control of the amines themselves, this chapter addresses certain aspects of their nitrosation. Occurrence of and exposure to the parent compounds are discussed in the chapters on the individual amines.

OCCURRENCE

N-Nitroso compounds form readily from a variety of amine- and amide-type compounds and nitrosating agents. The amines can be primary (Scanlan, 1975; Tannenbaum *et al.*, 1979), secondary (Mirvish, 1975; Scanlan, 1975), or tertiary (Lijinsky *et al.*, 1972; Ohshima and Kawabata, 1978). The nitrosating species can be derived from nitrite salts or nitrous acid, oxides of nitrogen--nitric oxide, nitrogen dioxide, dinitrogen trioxide, and dinitrogen tetraoxide (Challis and Kyrtopoulos, 1979; Challis *et al.*, 1978), or nitro compounds($O-NO_2$, $N-NO_2$, $C-NO$) (Fan *et al.*, 1978), or by transnitrosation from nitroso compounds (O-NO, N-NO, and C-NO) (Burglass et al., 1975). Depending on the reactants and the presence of catalysts, N-nitroso compound formation can occur at acidic, neutral, or alkaline pH or in organic media. Because N-nitroso compounds can be formed so readily from such a variety of widely distributed precursors, it is not surprising that low levels of N-nitroso compounds are ubiquitous in the environment. In fact, nitrosamines should be expected wherever amines are present.

Rapid Nitrosation of Amines with Oxides of Nitrogen

Primary, secondary, and tertiary amines are more readily nitrosated by nitrogen oxides such as nitric oxide (NO), nitrogen dioxide (NO_2), and dinitrogen tetroxide (N_2O_4) under mild nonacidic conditions than by aqueous nitrous acid (HNO_2). Deaminated products such as N-nitrosamines and, in some circumstances, N-nitramines are thus produced.

Several oxides of nitrogen, e.g., nitric oxide, nitrogen dioxide, dinitrogen trioxide (N_2O_3), and dinitrogen tetroxide, react with amines under mild conditions to form deaminated products, N-nitrosamines, and, in some instances, N-nitroamines. Generally, these reactions occur much more readily than do conventional nitrosations of amines involving nitrous acid. The weakest reagent is nitric oxide, which reacts slowly with secondary amines under anaerobic solutions to produce N-nitrosamines (Challis and Kyrtopoulos, 1979; Drago et al., 1961).These reactions are catalyzed, however, by oxygen (Drago *et al.*, 1961), iodine (Challis and Outram, 1979), hydrogen iodide (Challis *et al.*, 1978), metal salts such as zinc iodide, cuprous chloride, cupric chloride, ferrous chloride, ferric nitrate (Challis *et al.*, 1978), and silver nitrate (Challis and Outram, 1978). In many instances the reactions are complete in less than 20 minutes. The catalysis by oxygen involves nitrogen dioxide formation, and that by iodine, hydrogen iodide, and with metal iodides the formation of nitrosyl iodide. Catalysis by other metal salts, however, can involve oxidation of the amine to either a radical (R_2N) or radical cation (R_2N^+), which then combines rapidly with nitric oxide (Challis *et al.*, 1978). This mechanism has been proven for silver iodide salts.

Amine nitrosations by nitrogen dioxide ($\rightleftharpoons N_2O_4$) and nitric oxice plus nitrogen dioxide ($\rightleftharpoons N_2O_3$) are very rapid and probably proceed upon encounter (Challis and Kyrtopoulos, 1978). Such reactions have been examined in the gas phase in connection with smoking (Neurath *et al.*, 1976; Spincer and Westcott, 1976) and atmospheric pollution (Bretschneider and Matz, 1973; Hanst *et al.*, 1977; Pitts *et al.*, 1978; Tuazon *et al.*, 1978). The reactions also have application in chemical synthesis (Lovejoy and Vosper, 1968; White, 1955; White and Feldman, 1957).

More recent work has demonstrated that dinitrogen trioxide and dinitrogen tetroxide are also powerful nitrosating agents in aqueous solution; a wide range of primary and secondary amines form high yields of deaminated and *N*-nitroso products, respectively, in approximately 3 minutes when reacted with gaseous dinitrogen tri- and tetroxide under nonacidic conditions (Challis and Kyrtopoulos, 1977; Drago *et al.*, 1961). With dinitrogen tetroxide, small amounts of *N*-nitramine form concurrently. These reactions suggest that both dinitrogen trioxide and dinitrogen tetroxide exist in two tautomeric forms of different reactivity. With dilute (2-500 ppm) nitrogen dioxide, *N*-nitroamine formation becomes as significant as *N*-nitrosamine formation (Challis and Goff, 1981, personal communication). These reactions are strongly catalyzed by β-substituted alcohols such as ethylene glycol, alkanolamines, and carbohydrates (Challis *et al.*, 1980).

Industrial Exposure. The people with the largest daily exposure to preformed *N*-nitrosamines are factory workers in a variety of industries. The highest level of chronic exposure to *N*-nitrosodimethylamine (NDMA) is incurred by tanners (Rounbehler *et al.*, 1979), especially by those who work in the wet tanning area. NDMA has been found in five of five tanneries studied, at levels varying from a low of 23 $\mu g/m^3$ to high of 47 $\mu g/m^3$. The daily human exposure from this source can be as high as 440 μg. In addition, *N*-nitrosomorpholine (NMOR) has been shown to be present at a level of 2.0 $\mu g/m^3$ in the finishing area where the surfaces of the hides are chemically doped.

The curing and extrusion areas of rubber tire factories also have been shown to contain NMOR, at levels of 0.5 to 27 $\mu g/m^3$ (Fajen *et al.*, 1979). The daily human exposure at these levels is 50-250 μg.

NMOR probably arises as a trace contaminant in bismorpholincarbamylsulfenamide, which is used as an accelerator. *N*-nitrosodiphenylamine (NDPhA) has also been found at the 0.2-47.0 μg/m^3 level in a factory that manufactures it for use in tires.

NDMA was reported at the site of a rocket fuel factory where unsymmetrical dimethylhydrazine was being manufactured from NDMA (Fine *et al.*, 1976; 1977a,c). There, levels of NDMA in air varied from 2 to 36 μg/m^3. The average daily NDMA intake of workers ranged from 10 to 50 μg.

Nitrosamines have also been found in pesticides (Cohen *et al.*, 1978; Fine *et al.*, 1977b), industrial wastewater (Cohen and Bachman, 1978; Fine *et al.*, 1977a), and deionized water (Cohen, 1977; Fiddler *et al.*, 1977).

Inhalation. Tobacco smoke has been shown to contain a variety of nitrosamines, including *N*-nitrosonornicotine (NNN), *N*-nitrosoanatabine (NALB), 4-(*N*-methyl-*N*-nitrosamino)-1(3-pyridyl)-1-butanone (NNK), NDMA, and *N*-nitrosopyrrolidine (NPYR). Substances identified in a commercial filter cigarette include the following (average levels are provided): NNN - 310 ng/cigarette, NALB - 150 ng/cigarette, NNK - 370 ng/cigarette, NDMA - 5.7 ng/cigarette, and NPYR - 5.1 ng/cigarette (Brunnemann and Hoffmann, 1978; Hoffmann *et al.*, 1980). The total nitrosamine body intake for a person smoking 20 cigarettes per day is approximately 16.8 μg.

Volatile nitrosamines, including NDMA, NMOR, and N-nitrosodiethylamine (NDEA), have been found in the interiors of new automobiles (Rounbehler et al., 1980). Average levels were NDMA-0.3 $\mu g/m^3$, NMOR - 0.67 $\mu g/m^3$, and NDEA - 0.11 $\mu g/m^3$. The total body burden after a 60-minute exposure has been estimated to be 0.6 μg (Fine et al., 1980).

Ingestion. Ingestion is the most widely studied route of human exposure to N-nitrosamines, and studies are well documented for a variety of foodstuffs including bacon, cured meat, meatloaf, salami, ham, tinned meat, sausage, poultry, smoked and cooked fish, shellfish, cheese, and yogurt (International Agency for Research on Cancer, 1978). Only in cooked bacon and ham does the nitrosamine level usually exceed 1 μg/kg.

Until recently, the largest exposure to ingested nitrosamines came from beer (Spiegelhalder et al., 1979). A study of beer from Australia, France, Greece, Holland, Ireland, Japan, Mexico, the Philippines, the United Kingdom, and the United States showed an average NDMA content of 2.8 μg/liter (Goff and Fine, 1979). A person drinking three cans of beer was therefore exposed to approximately 8.4 μg of NDMA. Of course, a person drinking a beer containing more than the average amount of NDMA was exposed to proportionally more of the substance. Webb and Gough (1980) in the United Kingdom and Stephany and Schuller (1980) in the Netherlands have shown that more than 80% of the ingested nitrosamines in their countries came from beer.

Scotch whisky contains 0.3-2.0 µg/liter of NDMA; a single 20-ml drink contains only 0.03 µg. Wines, liqueurs, gins, brandies, vodkas, and rums have not been found to contain volatile nitrosamines (Goff and Fine, 1979).

Dermal Exposure. *N*-Nitrosodiethanolamine (NDElA) has been found in cutting fluids (Fan *et al.*, 1977b), cosmetics, shampoos, and lotions (Fan *et al.*, 1977a). Edwards *et al.* (1979) showed that NDElA was present in the urine of a person wearing an NDElA-contaminated cosmetic purchased over the counter. Approximately 1.7% of the NDElA applied to the skin for 8 hours appeared as NDElA in urine over a 21-hour period. Recent studies by the Food and Drug Administration (Wenninger, 1979) indicated that many U.S. cosmetics are still contaminated with NDElA.

In vivo. *N*-Nitrosamines, especially NDMA, have been observed *in vivo* in humans (Fine *et al.*, 1977b; Kowalski *et al.*, 1980; Tannenbaum, 1980). Approximately 30% of the people tested had NDMA present for some of the day. The data are based on the analysis of a few milliliters of blood, urine, or feces with typical NDMA levels of 0.01 to 0.1 µg/liter.

Analyses at these low levels are extremely difficult because of the problem of false artifact formation during sample preparation. Tannenbaum (1980) has estimated that daily *in vivo* exposure to NDMA could be as high as 1,000 µg assuming that current analytical methods are valid.

Assessment of Relative Exposures. Fine (1980) compared the average human intake of preformed nitrosamines, in terms of relative exposure of the U.S. population, by estimating body intake and then by adjusting that amount for the number of years a population would be exposed and the number of people who would be exposed. Table 1-1 shows cigarette smoking to be at the top of the list, followed by four exposure sources of approximately equal importance: new car interiors, beer, cosmetics, and bacon. Scotch whisky is at the bottom of the list. If *in vivo* formation were included, an individual's exposure could be as high as 1,000 μg.

In principle, exposure should also take into account the type of nitrosamine, its route of exposure, its relative potency, and other factors. Thus, although both NDMA and NMOR have been shown to be potent carcinogens at low doses in animals (International Agency for Research on Cancer, 1978; Moiseev and Benemansky, 1975; Shank and Newberne, 1976), much of the biological data needed to make an extrapolation to humans are unavailable. Even if there were such data, the question of how to extrapolate them from animals to humans would still be the subject of intense scientific debate. Nevertheless, it is important to appreciate just how significant these unknown factors may be. In rats, for example, if proper account were taken of the exposure route and if the relative potency of NPYR and NDElA versus NDMA were known (and even this minor extrapolation is subject to dispute), then the relative rankings of the importance of exposure to car interiors versus exposure to cosmetics would differ by a hundredfold.

Table 1-1

A Comparison of Average Human Intake of Nitrosamines In Terms of Relative Exposure of the U.S. Population

Exposure	Nitrosamine*	Prime Exposure Route	Average Measured Concentration	Body Intake, ng/kg/day	Average Number of Years Exposed	Population Exposed, 10^6	Relative Intake of Exposed Population Arbitrary Unit
Smoking[a]	NNN	Inhalation	310 ng/cigarette	240	40	50	1,500
	NALB	Inhalation	150 ng/cigarette				
	NNK	Inhalation	370 ng/cigarette				
	NDMA	Inhalation	5.7 ng/cigarette				
	NPYR	Inhalation	5.1 ng/cigarette				
New Car Interior[b]	NDMA	Inhalation	0.3 $\mu g/m^3$	9.2	23	200	130
	NMOR	Inhalation	0.67 $\mu g/m^3$				
	NDEA	Inhalation	0.11 $\mu g/m^3$				
Beer[c]	NDMA	Ingestion	2.8 μg/liter	15	40	50	90
Cosmetics[d]	NDElA	Dermal	11 μg/g	6.3	50	70	70
Cooked bacon[e]	NPYR	Ingestion	5 μg/g	2.4	70	100	50
Scotch whisky[f]	NDMA	Ingestion	0.97 μg/liter	0.4	40	20	1

*NNN = N-nitrosonornicotine
NALB = N-nitrosoanatabine
NNK = 4-(N-methyl-N-nitrosamino)-1(3-pyridyl)-1-butanone
NDMA = N-nitrosodimethylamine
NPYR = N-nitrosopyrrolidine
NMOR = N-nitrosomorpholine
NDElA = N-nitrosodiethanolamine.

[a] Average NNN, NALB, and NNK levels for a commercial cigarette with filter were obtained from Hoffmann *et al*. (1979). NMDA and NPYR levels were obtained from Brunnemann and Hoffmann (1978). The Surgeon General's Report on Smoking and Health (U.S. Department of Health, Education, and Welfare, 1979) was used to estimate that 33-35% of the U.S. population over the age of 18 smoke cigarettes.

[b] The average levels of nitrosamines in new car interiors was: NDMA, 0.30 $\mu g/m^3$; NDEA 0.11 $\mu g/m^3$; and NMOR, 0.67 $\mu g/m^3$ (Rounbehler *et al*., 1980). It was assumed that the average duration of exposure per day is 60 minutes for 200 x 10^6 people and that a new car is purchased every 3 years.

[c] The average NDMA content for U.S. beer was calculated by averaging the data from 18 samples analyzed by Goff and Fine (1979). The average U.S. beer consumption was assumed to be 132.475 liters/yr, for an average NDMA intake of 15 ng/kg/day. It was assumed that 50 x 10^6 people drink this quantity of beer daily.

[d] The average NDELA level in cosmetics was calculated by averaging the seven data points in Fan *et al*. (1977a). If a woman uses 2 g of cosmetics per day, then 22,000 ng of NDELA would be applied to the skin, 2% of which would penetrate the skin (Edwards *et al*., 1979). The average daily intake of NDELA would therefore be 6.3 ng/kg/day.

[e] The 1979, U.S. production of bacon was 2.85 kg/person. The USDA requires that cooked bacon shall contain less than 10 $\mu g/kg$ of NPYR; it was assumed that the average NPYR level in U.S. bacon is half this amount, or 5 $\mu g/kg$. It was assumed that half the U.S. population consumes bacon, and that these people eat bacon for their entire lives.

[f] The average NDMA content of scotch whisky was calculated by averaging the data from seven samples analyzed by Goff and Fine (1979). It was assumed that 20 x 10^6 people drink 30 ml per day, producing an average NDMA intake of 0.4 ng/kg/day.

CONTROL

Among the compounds evaluated in this report and in its companion report on aromatic amines, triethanolamine, morpholine, trifluralin, and oryzalin have all been shown to be contaminated with nitrosamines (Fine, 1980). When trifluralin was first tested for the presence of N-nitrosamines, the impurity N-nitrosodipropylamine was found in concentrations of 195,000 ppb (Fine, 1980). The contamination occurred during manufacture where ring nitration with nitric and sulfuric acids was followed by the addition of dipropylamine. The manufacturing process was modified as soon as the manufacturer realized that the product was contaminated; the nitrosamine impurity was quickly reduced to below 1,000 ppb.

NDMA was found at levels as high as 640,000 ppb in the dimethylamine salts of the herbicide 2,3,6-trichlorobenzoic acid (Fine, 1980). The manufacturer eliminated the problem by switching the packaging from metal (sodium-nitrite-treated) cans to plastic-lined cans. The U.S. Environmental Protection Agency now requires manufacturers to provide information on the nitrosamine levels in pesticide products.

Shortly after publication of data showing that cutting fluids were contaminated with as much as parts per hundred impurities of NDElA, the National Institute for Occupational Safety and Health (1976) issued a Current Intelligence Bulletin. Within a few months, the industry began advertising "nitrosamine-free" cutting fluids in its trade journals. The problem was solved by ensuring that the product did not contain both sodium nitrite and triethanolamine in the same formulation.

The NDMA impurity in beer was found to arise from N-nitrosation by nitrogen oxides (NO_x) during drying of malt. Regulatory pressure from the U.S. Food and Drug Administration (FDA) to reduce the NDMA level in beer to less than 5 ppb (5 μg/liter) has forced the industry to develop short-term solutions. These include the use of gas burners with lower NO_x output and an increase in the acidity of the malt by sulfuring (burning solid sulfur and sweeping the malt beds with sulfur dioxide gases). Sulfuring may not be a viable long-term solution in view of sulfur dioxide emission regulations.

In the tire industry, human exposure to NMOR in some factories has been reduced tenfold by increasing the ventilation capacity tenfold. NMOR is derived from various accelerators, which decompose to release morpholine. The industry is investigating the use of non-morpholine-based accelerators.

The nitrosamine levels in pork products, especially in cooked bacon, have been lowered over the past several years because of pressure from the U.S. Department of Agriculture (USDA) and the FDA to lessen the amount of nitrite added. Ascorbate has thus been added to the bacon cures. USDA currently limits the NPYR level in cooked bacon to less than 10 ppb.

During the past few years, it has been demonstrated that reductions in nitrosamine contamination can be achieved at minimum cost, as manufacturers become aware of the problem. Thus, the most effective control technology, is the widespread dissemination of accurate knowledge about the formation of nitrosamines. Manufacturers and users of amines and nitrosating agents should be made aware that their processes and products are probably contaminated but that the extent of the contamination can be lessened.

References

Bretschneider, K., and J. Matz. 1974. Nitrosamines in the atmospheric air and in the air at the places of employment. Arch. Geschwulstforsch. 43:36-41. (in English) [Chem. Abs. 81:110761d, 1974]

Brunnemann, K.D., and D. Hoffman. 1978. Chemical studies on tobacco smoke LIX. Analysis of volatile nitrosamines in tobacco smoke and polluted indoor environments. Pp.343-356 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Buglass, A.J., B.C. Challis, and M.R. Osborne. 1975. Transnitrosation and decomposition of nitrosamines Pp. 94-100 in P. Bogovski and E.A. Walker, eds. N-Nitroso Compounds in the Environment. IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon.

Challis, B.C., and S.A. Kyrtopoulos. 1977. Rapid formation of carcinogenic N-nitrosamines in aqueous alkaline solutions. Br. J. Cancer 35:693-696.

Challis, B.C., and S.A. Kyrtopoulos. 1978. The chemistry of nitroso compounds. Part 12. The mechanism of nitrosation and nitration of aqueous piperidine by gaseous dinitrogen tetraoxide and dinitrogen trioxide in aqueous alkaline solutions. Evidence for the existence of molecular isomers of dinitrogen tetraoxide and dinitrogen trioxide. J. Chem. Soc. Perkin Trans. 2(12):1296-1302.

Challis, B.C., and S.A. Kyrtopoulos. 1979. The chemistry of nitroso-compounds. Part 11. Nitrosation of amines by the two-phase interaction of amines in solution with gaseous oxides of nitrogen. J. Chem. Soc. Perkin Trans. 1(2):299-304.

Challis, B.C., and J.R. Outram. 1978. Rapid formation of N-nitrosamines from nitric oxide in the presence of silver (I) salts. J. Chem. Soc. Chem. Commun., No. 16:707-708. [Chem. Abs. 90:38228b, 1979]

Challis, B.C., and J.R. Outram. 1979. The chemistry of nitroso compounds. Part 15. Formation of N-nitrosamines in solution from gaseous nitric oxide in the presence of iodine. J. Chem. Soc. Perkin Trans. 1, No. 11:2768-2775. [Chem. Abs. 92:180240j, 1980]

Challis, B.C., A. Edwards, R.R. Hunma, S.A. Kyrtopoulos, and J.R. Outram. 1978. Rapid formation of N-nitrosamines from nitrogen oxides under neutral and alkaline conditions. Pp. 127-142 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Challis, B.C., J.R. Outram, and D.E.G. Shuker. 1980. New pathways for the rapid formation of N-nitrosamines under neutral and alkaline conditions. In E.A. Walker, M. Castegnaro *et al.*, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence. IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon.

Cohen, J.B. 1977. Reconnaissance of environmental levels of nitrosamines in the central United States, U.S. Government Report No. EPA-330/1-77-001. U. S. Environmental Protection Agency, Office of Enforcement, National Enforcement Investigations Center, Denver, Colo.

Cohen, J.B., and J.D. Bachman. 1978. Measurement of environmental nitrosamines. Pp. 357-372 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects on N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Cohen, S.Z., G. Zwseig, M. Law, D. Wright, and W.H. Bontoyan. 1978. Analytical determination of N-nitroso compounds in pesticides by the United States Environmental Protection Agency -- A preliminary study. Pp. 333-342 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Drago, R.S., R.O Ragsdale, and D.P. Eyman. 1961. A mechanism for the reaction of diethylamine with nitric oxide. J. Am. Chem. Soc. 83:4337-4339.

Edwards, G.S., J.G. Fox, P. Policastro, U. Goff, W.H. Wolf, and D.H. Fine. 1979. Volatile nitrosamine contamination in laboratory animal diets. Cancer Res. 39:1857-1858.

Fajen, J.M., G.A. Carson, D.P. Rounbehler, T.Y. Fan, R. Vita, E.U. Goff, M.H. Wolf, G. S. Edwards, D.H. Fine, V. Reinhold, and K. Biemann. 1979. N-nitrosamines in the rubber and tire industry. Science 205:1262-1264.

Fan, T.Y., U. Goff, L. Song, D.H. Fine, G.P. Arsenault, and K. Biemann. 1977a. N-Nitrosodiethanolamine in cosmetics, lotions and shampoos. Food Cosmet. Toxicol. 15:423-430.

Fan, T.Y., J. Morrison, D.P. Rounbehler, R. Ross, D.H. Fine, W. Miles, and N.P. Sen. 1977b. N-nitrosodiethanolamine in synthetic cutting fluids: A part-per-hundred impurity. Science 196:70-71.

Fan, T.Y., R. Vita, and H.H. Fine. 1978. C-nitro compounds: A new class of nitrosating agents. Toxicol. Lett. 2:5-10.

Fiddler, W., J.W. Pensabene, R.C. Doerr, and C.J. Dooley. 1977. The presence of dimethyl and diethyl-nitrosamines in deionized water. Food Cosmet. Toxicol. 15:441-443.

Fine, D.H. 1980. N-Nitroso compounds in the environment. Pp. 39-123 in J.N. Pitts, Jr. and R. Metcalf, eds. Advances in Environmental Science and Technology, Vol. 10. Wiley & Sons, New York.

Fine, D.H., D.P. Rounbehler, E.D. Pellizzari, J.E. Bunch, R.W. Berkeley, J. McCrae, J.T. Bursey, E. Sawicki, K. Krost, and G.A. DeMarrais. 1976. N-nitrosodimethylamine in air. Bull. Environ. Contam. Toxicol. 15:739-746.

Fine, D.H., R. Ross, D.P. Rounbehler, A. Silvergleid, and L. Song. 1977a. Formation in vivo of volatile N-nitrosamines in man after ingestion of cooked bacon and spinach. Nature (London) 265:753-755.

Fine, D.H., D.P. Rounbehler, T. Fan, and R. Ross. 1977b. Human exposure to N-nitroso compounds in the environment. Pp. 293-307 in H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds. Origins of Human Cancer, Book A. Cold Spring Harbor Conferences on Cell Proliferation, Vol. 4. Cold Spring Harbor, N. Y.

Fine, D.H., D.P. Roundbehler, A. Rounbehler, A. Silvergleid, E. Sawicki, E. Krost, and G.A. DemMarrais. 1977c. Determination of dimethylnitrosamine in the air, water, and soil by thermal energy analysis: Measurements in Baltimore, Md. Environ. Sci. Technol. 11:581-584.

Goff, E.U., and D.H. Fine. 1979. Analysis of volatile N-nitrosamines in alcoholic beverages. Food Cosmet. Toxicol. 17:569-573.

Hanst, P.L., J.W. Spence, and M. Miller. 1977. Atmospheric chemistry of N-nitroso dimethylamine. Environ. Sci. Technol. 11:403-405.

Hoffmann, D., J.D. Adams, J.J. Piade, and S.S. Hecht. 1980. Analysis of volatile and tobacco specific nitrosamines in tobacco products. In E.A. Walker, M. Castegnaro *et al.*, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence. IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon.

International Agency for Research on Cancer. 1978. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 17. Some N-nitroso compounds. International Agency for Research on Cancer, Lyon. 365 pp.

Kowalski, B., C.T. Miller, and N.P. Sen. 1980. Studies on the *in vivo* formation of nitrosamines in rats and humans after ingestion of various meals. In E.A. Walker, M. Castegnaro *et al.*, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence. IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon.

Lijinsky, W., L. Keefer, E. Conrad, and R. van de Bogart. 1972. The nitrosation of tertiary amines and some biological implications. J. Natl. Cancer Inst. 49:1239-1249.

Lovejoy, D.L., and A.J. Vosper. 1968. Dinitrogen trioxide. Part VI. The reactions of dinitrogen trioxide with primary and secondary amines. J. Chem. Soc. A.:2325-2328.

Mirvish, S.S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, *in vivo* occurrence. Toxicol. Appl. Pharmacol. 31:325-351.

Moiseev, G.E., and V.V. Benemanskii. 1975. Carcinogenic activity of low concentrates of nitrosdimethylamine in inhalation. Vopr. Onkol. 21(6):107-109. [Chem. Abs. 83:173618z, 1975]

National Institute for Occupational Safety and Health. 1976. Current Intelligence Bulletin 15: Nitrosamines in Cutting Fluids. U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Rockville, Md. October 6, 1976.

Neurath, G.B., M. Dunger, and F.G. Pein. 1976. Nitrosation of nornicotine and nicotine in gaseous mixtures and aqueous solutions. Pp. 227-236 in E.A. Walker, P. Bogovski, and L. Griciute, eds. Environmental N-Nitroso Compounds: Analysis and Formation. IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon.

Ohshima, H., and T. Kawabata. 1978. Mechanisms of N-nitrosodimethylamine formation from trimethylamine and trimethylaminoxide. Pp. 143-153 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Pitts, J.N., Jr., D. Grosjean, K. van Cawvenberghe, J.P. Schmid, and D.R. Fitz. 1978. Photooxidation of aliphatic amines under simulated atmospheric conditions: Formation of nitrosamines, nitramines, amides, and photochemical oxidant. Environ. Sci. Technol. 12:946-953.

Rounbehler, D.P., I.S. Krull, E.U. Goff, K.M. Mills, J. Morrison, G.S. Edwards, D.H. Fine, J.M. Fajen, G.A. Carson, and V. Reinhold. 1979. Exposure to N-nitrosodimethylamine in a leather tannery. Food Cosmet. Toxicol. 17:487-491.

Rounbehler, D.P., J. Reisch, and D.H. Fine. 1980. Nitrosamines in new motor cars. Food Cosmet. Toxicol. 18:147-151.

Scanlan, R.A. 1975. N-nitrosamines in foods. Crit. Rev. Food Technol. 5:357-402.

Shank, R.C., and P.M. Newberne. 1976. Dose-response study of the carcinogenicity of dietary sodium nitrite and morpholine in rats and hamsters. Food Cosmet. Toxicol. 14:1-8.

Spiegelhalder, B., G. Eisenbrand, and G. Preussmann. 1979. Contamination of beer with trace quantities of N-nitrosodimethylamine. Food Cosmet. Toxicol. 17:29-31.

Spincer, D., and D.T. Westcott. 1976. Formation of nitrosodimethylamine in smoke from cigarettes manufactured from different tobacco types. Pp. 133-139 in E.A. Walker, P. Bogovski, and L. Griciute, eds. Environmental N-Nitroso Compounds: Analysis and Formation. IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon.

Stephany, R.W., and P.L. Schuller. 1980. Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in The Netherlands using the duplicate portion sampling technique. Oncology 37:203-210.

Tannenbaum, S.R. 1980. A model for estimation of human exposure to endogenous N-nitrosodimethylamine. Oncology 37:232-235.

Tannenbaum, S.R., J.S. Wishnok, J.S. Hovis, and W.W Bishop. 1978. N-Nitroso compounds from the reaction of primary amines with nitrite and thiocyanate. Pp. 155-159 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Tuazon, E.C., A.M. Winer, R.A. Graham, J.P. Schmid, and J.N. Pitts, Jr. 1978. Fourier transform infrared detection of nitramines in irradiated amine-NO_x systems. Environ. Sci. Technol. 12:954-958.

U.S. Department of Health, Education, and Welfare. 1979. Smoking and Health: A Report of the Surgeon General. DHEW Pub. No. (PHS) 7-50066. U.S. Department of Health, Education and Welfare, Public Health Service, Office of the Assistant Secretary for Health, Office on Smoking and Health, Bethesda, Md. 1,136 pp.

Webb, K.S., and T.A. Gough. 1980. Human exposure to preformed environmental N-nitroso compounds in the U.K. Oncology 37:195-198.

Wenninger, J.A., 1979. FDA Progress Report - Nitrosamine Contamination of Cosmetic Products, March 20, 1979. Food and Drug Administration, Washington, D.C.

White, E.H. 1955. The chemistry of N-alkyl-N-nitrosamides I. Methods of preparation. J. Amer. Chem. Soc. 77:6008-6010.

White, E.H., and W.R. Feldman. 1957. The nitrosation and nitration of amines in alcohols with nitrogen tetroxide. J. Am. Chem. Soc. 79:5832-5833.

Chapter 2

GENERAL ANALYTIC METHODS

A variety of procedures for analyzing aliphatic amines are described in the literature. Among them are thin-layer chromatography (TLC), paper chromatography, gas chromatography (GC), and spectrophotometric procedures. TLC procedures are described by Churacek _et al_. (1972), Gruger (1972), Schwartz and Brewington (1967), and Wick _et al_. (1967). Paper chromatography of free amines is described by Slaughter and Uvgard (1971), and of various derivatives by Churacek _et al_. (1972). Much recent GC work on free amines has been performed by Gruger (1972), Jones (1963), Miller _et al_. (1973), Preston and Prankratz (1970), Thombropoulos (1979), and Wick _et al_. (1967). Other researchers (Golovnya, 1976; Kann _et al_., 1976; Knapp, 1979; Mosier _et al_., 1973; Neurath _et al_., 1966 and Singer and Lijinsky, 1976 a,b) have performed work on various derivatives.

Spectrophotometric procedures have been used by Burenko _et al_. (1977), Karweik and Meyers (1979), and Zalnierius (1974). Fong and Chang (1976 a,b) identified secondary amines by determining the increases in _N_-nitrosamine content following drastic nitrosation with 0.145 M sodium nitrite at pH 3.

Although this listing is by no means complete, it is indicative of the wide variety of procedures that have been used to analyze aliphatic amines. The most widely applied procedures involve either GC or TLC.

Gas Chromatography

Gas chromatography has been used in much of the recent analytic work on aliphatic amines . Direct GC separation and determination of amines without formation of derivatives is generally unsatisfactory. Interactions between strong bases and active sites on the supports frequently cause peak tailing; even "ghost" effects are sometimes observed. Coating with alkali, which is often recommended, is not entirely satisfactory. The use of graphitized carbon black, thermally treated in a hydrogen stream and coated with basic compounds such as tetraethylene pentamine, is claimed to eliminate adsorption effects and to give symmetrical peaks with amines (DiCorcia and Samperi, 1974). This method was successfully used to determine the presence of aliphatic amines in aqueous solutions. Miller *et al*. (1972) utilized the technique to identify dimethyl- and trimethylamines in fish, using an alkali flame-ionization detector (AFID).

The main progress in the GC analysis of amines has been through the development of suitable derivatives (Knapp, 1979). Using GC and a flame-ionization detector (FID), the trifluoroacetamides have been used to detect primary and secondary amines in fresh vegetables, preserves, mixed pickles, fish and fish products, bread, cheese, stimulants, and surface waters (Neurath *et al*., 1977).

Walle and Ehrsson (1970) have used heptafluorobutyryl derivatives in combination with electron-capture methods to detect picogram quantities of amino compounds. Heptafluorobutyrylimidazole has been recommended as a reagent for amines by Staab and Walther (1960).

With the object of restricting analysis to the naturally occurring nitrosatable amines, Singer and Lijinsky (1976a,b) chose the classic Hinsberg method of forming the _p_-toluenesulfonyl derivatives to separate the secondary amines from their accompanying primary and tertiary amines. Tertiary amines do not react, and the products of primary amines are soluble in alkali; thus, the secondary amine derivatives can be isolated easily. The _p_-toluenesulfonamides are readily separated by GC and have characteristic mass-spectrometric fragmentation patterns that facilitate their identification.

Numerous other derivatization methods have been proposed. For example, Gejvall (1974) analyzed the urethanes formed by reaction of amines with diethyl pyrocarbonate, using GC with AFID. The reaction of different isocyanates with amines from N,N'-di- and N,N',N'-trisubstituted ureas was studied by Nitsche _et al_. (1974) who found the _tert_-butyl and the 3-trifluoromethylphenylureas to be useful derivatives for GC analysis of primary and secondary amines.Electron-capture and nitrogen detectors, as well as mass spectrometry, have also been used with these derivatives.

Long-chain primary amines have been analyzed by GC, after conversion to their dimethylamine derivatives (Metcalfe and Martin, 1972); excellent separation was reported on silicone oil capillary columns.

Thin-Layer Chromatography

Thin-layer chromatography has been applied to the analysis of amines, often to a derivative selected for its particular properties, such as color or fluorescence. A few of the more important techniques are mentioned below.

Hydrochlorides of primary, secondary, and tertiary amines have been separated on buffered silica gel by Teichert *et al*. (1960) and could be detected in amounts ranging from 0.1 to 10.0 μg. Grasshoff (1965) carried out a similar separation on magnesium silicate layers. Ninhydrin has been used as a general reagent for the detection of primary amines, sodium nitroprusside for secondary amines, and Dragendorff reagent for tertiary amines.

Good separation and high sensitivity on silica gel plates were achieved by Neurath and Doerk (1964), using the red-colored 4'-nitroazobenzyl-(4)-amides, which permitted detection of less than 1 μg of amine.

Colorimetry generally detects the acylated derivatives of primary and secondary amines more reliably than it does the parent amines. Seiler and Weichmann (1965, 1967) were the first to take advantage of the fluorescing properties of the 1-dimethylaminonaphthalene-5-sulfonamides (dansyl derivatives) on silica gel. Under ultraviolet light (365 nm), as little as 10^{-10} mol is detectable.

Klimisch and Stadler (1974) described microquantitative determination of aliphatic amines by the formation of fluorescent derivatives with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. One advantage of this method is that the reagent itself does not fluoresce. The derivatives were separated by TLC on polyamide-11 foils.

The methods described above have been applied to a variety of matrices, including biological samples such as blood and urine (Karweik and Meyers, 1979; Tombropoulos, 1979); industrial chemicals (Zalnierius, 1974); air monitoring (Burenko _et al_., 1977; Jones, 1963); tobacco and tobacco condensates (Neurath _et al_., 1966; Singer and Lijinsky, 1976a,b); and various food products including fresh and saltwater fish, milk, tea, beer, wine, ham, frankfurters, cheese, and bread (Golovyna, 1976; Gruger, 1972; Kann _et al_., 1976; Miller _et al_., 1972; Singer and Lijinsky, 1976a).

Analysis of the specific chemicals discussed in this report will be found in the chapters on those compounds.

REFERENCES

Burenko, T.S., E.G. Zhuravlev, and T.A. Miklashevich. 1977. Determination of morpholine in air. Gig. Tr. Prof. Zabol. No. 3:55-56. [Chem. Abs. 86:194286s, 1977]

Churacek, J., H. Pechová, D. Tocksteinová, and Z. Zikova. 1972. Separation and identification of primary and secondary aliphatic amines as p-(N,N-dimethylamino)-benzene-p'-azobenzamides by paper and thin layer chromatography. J. Chromatogr. 72:145-152.

DiCorcia, A., and R. Samperi. 1974. Gas chromatographic determination at the parts-per-million level of aliphatic amines in aqueous solutions. Anal. Chem. 46:977-981.

Fong, Y.Y., and W.C. Chan. 1976a. Effect of ascorbate on amine-nitrite carcinogenicity. Pp. 461-464 in E.A. Walker, P. Bogovski, and L. Griciute, eds. Environmental N-Nitroso Compounds: Analysis and Formation. IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon.

Fong, Y.Y., and W.C. Chan. 1976b. Reduction of nitrosodimethylamine content of Cantonese salt fish. Pp. 465-471 in E.A. Walker, P. Bogovski, and L. Griciute, eds. Environmental N-Nitroso Compounds: Analysis and Formation. IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon.

Gejvall, T. 1974. Gas chromotographic analysis of amines separated as urethane derivatives. J. Chromatogr. 90:157-161.

Golovnya, R.V. 1976. Analysis of volatile amines contained in foodstuffs as possible precursors of N-nitroso compounds. Pp. 237-245 in E.A. Walker, P. Bogovski, and L. Griciute, eds. Environmental N-Nitroso Compounds: Analysis and Formation. IARC Scientific Publications No. 14. International Agency for Research on Cancer, Lyon.

Grasshoff, H. 1965. Dünnschicht-Chromatographie von Aminen. J. Chromatogr. 20:165-167.

Gruger, E.H., Jr. 1972. Chromatographic analyses of volatile amines in marine fish. J. Agric. Food. Chem. 20:781-785.

Jones, L.R. 1963. The determination of 2-nitropropane in air. Am. Ind. Hyg. Assoc. J. 24:11-16.

Kann, J., O. Tauts, K. Raja, and R. Kalve. 1976. Nitrosamines and their precursors in some Estonian foodstuffs. Pp. 385-394 in E.A. Walker, P. Bogovski, and L. Griciute, eds. Environmental N-Nitroso Compounds Analysis and Formation. IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon.

Karweik, D.H., and C.H. Meyers. 1979. Spectrophotometric determination of secondary amines. Anal. Chem. 51:319-320.

Klimisch, H.-J., and L. Stadler. 1979. Mikroquantitative Bestimmung von Aliphatischen Aminen mit 7-Chlor-4-nitrobenzo-2-oxa-1,3-diazol. J. Chromatogr. 90:141-148.

Knapp, D.R. 1979. Handbook of Analytical Derivatization Reactions. Wiley and Sons, New York. 741 pp.

Metcalfe, L.D., and R.J. Martin. 1972. Gas chromatography of positional isomers of long chain amines and related compounds. Anal. Chem. 44:403-405.

Miller, A., III, R.A. Scanlan, J.S. Lee, and L.M. Libbey. 1972. Quantitative and selective gas chromatographic analysis of dimethyl- and trimethylamine in fish. J. Agric. Food Chem. 20:709-711.

Miller, A., III, R.A. Scanlan, L.M. Libbey, H. Petropakis, and A.F. Anglemier. 1973. Quantitative determination of dimethyl- and trimethylamine in fish protein concentrate. J. Agric. Food Chem. 21:451-453.

Mosier, A.R., C.E. Andre, and F.G. Viets, Jr. 1973. Identification of aliphatic amines volatilized from cattle feedyard. Environ. Sci. Technol. 7:642-645.

Neurath, G., and E. Doerk. 1964. Identifizierung und quantitative Bestimmung einzelner primarer und sekundarer Amine aus Gemischen als 4'-Nitro-azobenzolcarbonsaure-(4)-amide. Chem. Ber. 97:172-178.

Neurath, G.B., M. Dunger, J. Gewe, W. Luttich, and H. Wichern. 1966. Untersuchung der Fluchtigen Basen des Tabakrauches. Beitr. Tabakforsch. 3:563-569.

Neurath, G.B., M. Dunger, F.G. Pein, D. Ambrosius, and O. Schreiber. 1977. Primary and secondary amines in the human environment. Food Cosmet. Toxicol. 15:275-282.

Nitsche, I., F. Selenka, and K. Ballschmiter. 1974. Zum Metabolismus von Dialkyldithiocarbamaten. 1. Mitt. Bestimmung der beim Abbay entstehenden Amine durch Umsetzung mit Isocyanaten und gaschromatographische Identifizierung der gebildeten Harnstoffderivate. J. Chromatogr. 94:65-73.

Preston, S.T., Jr., and R. Pankratz. 1970. A Guide to the Analysis of Amines by Gas Chromatography. Polyscience Corporation, Niles, Ill.

Schwartz, D.P., and C.R. Brewington. 1967. Methods for the isolation and characterization of constituents of natural products. V. Separation of 2,6,dinitrophenylhydrazone pyruvamides into classes and resolution of the individual members. Michrochem. J. 12:547-554.

Seiler, N., and M. Wiechmann. 1965. Zum Nachweiss von Aminen im 10^{10}-Mol-Mass-stab. Trennung von 1-Dimethylaminonaphthalin-5-sulfonsaure-amiden auf Dunnschicht chromatogrammen. Experientia 21:203-204.

Seiler, N., and M. Wiechmann. 1967. Zur chromatographie einiger 1-Dimethylamino-naphthalin-5-sulfony-Derivate auf Kieselgel G-Schichten. J. Chromatogr. 28:351-362.

Singer, G.M., and W. Lijinsky. 1976a. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. J. Agric. Food Chem. 24:550-553.

Singer, G.M., and W. Lijinsky. 1976b. Naturally occurring nitrosatable amines: II. Secondary amines in tobacco and cigarette smoke condensate. J. Agric. Food Chem. 24:553-555.

Slaughter, J.C., and A.R.A. Uvgard. 1971. Volatile amines of malt and beer. J. Inst. Brew. London 77:446-450. [Chem. Abs. 76:12811x, 1972]

Staab, H.A., and G. Walther. 1960. N-(Trifluoroacetyl)-imidazole. Angew. Chem. 72:35.

Teichert, K., E. Mutschler, and H. Rochlmeyer. 1960. Beitrage zur analytischen Chromatrographie. (2. Mitteilung). Dtsch. Apoth. Ztg. 100:283-286.

Tombropoulos, E.G. 1979. Micromethod for the gas chromatographic determination of morpholine in biological tissues and fluids. J. Chromatogr. 164:95-99.

Walle, T., and H. Ehrsson. 1970. Quantitative gas chromatographic determination of picogram quantities of amino and alcoholic compounds by electron capture detection. I. Preparation and properties of the heptafluorobutyryl derivatives. Acta Pharm. Suec. 7:389-406. [Chem. Abs. 73:123556a, 1970]

Wick, E.L., E. Underriner, and E. Paneras. 1967. Volatile constituents of fish protein concentrate. J. Food. Sci. 32:365-370.

Zalnierius, J. 1974. Photometric determination of triethanolamine in aqueous solutions. Nauch. Konf. Khim.-Anal. Pribalt Resp. B. SSR [Tezisy Dokl.], 1st, p. 125-128. [Chem. Abs. 86:21401x, 1977]

Chapter 3

EPIDEMIOLOGY

There is much less epidemiologic evidence pertaining to the adverse health effects caused by aliphatic amines than exists for aromatic amines. Although an early association was established for the induction of bladder cancer and exposure to certain aromatic amines, no such association has been shown for aliphatic amines, perhaps because the aliphatics are much less commonly used or are less toxic.

The most concern about the danger of aliphatic amines derives from the ease and rapidity with which they react to form various N-nitrosamines. Chapter 1 contains discussions about the reactions themselves; this chapter reviews what little is known about the epidemiologic aspects of exposure to aliphatic amines and their associated N-nitrosamines.

2-Nitropropane

On the basis of data from studies in animals, the National Institute for Occupational Safety and Health (NIOSH) recommended in 1976 that 2-nitropropane (2-NP) be regarded as if it were a carcinogen in humans. A retrospective cohort study of mortality among persons who manufacture 2-NP was conducted by the International Minerals & Chemical Corporation (1979). On the basis of the data provided by the company, the investigators concluded that there was not "any unusual cancer or other disease mortality pattern among this group of workers, either before or after the beginning of 2-NP production in 1955. However, both because the cohort is small and because the period of latency (the time between first exposure and observation) is for most relatively short, one cannot conclude from these data that 2-NP is non-carcinogenic in humans."

Among this group of 1,815 workers, 180 were deceased and seven had died from "sarcomatous" cancers. Because the sarcomas occurred at a number of sites and involved a variety of tissue types, no comparison was possible. Furthermore, because none of these cases of cancer occurred among workers judged to be directly or indirectly exposed to 2-NP, it seems unlikely that 2-NP was related to the disease.

Hexamethylenetetramine

For several decades, hexamethylenetetramine has been reported to cause "rubber itch," a dermatitis in rubber workers; (Heyhyrst and Kober, 1924). The substance also appears on NIOSH's list of suspected carcinogens (National Institute for Occupational Safety and Health, 1976).

Among a group of rubber tire manufacturers, 12 skin cancers occurred, as compared to 1.9 expected (Monson and Fine, 1978). On the basis of these data, it is not possible to determine whether the amine caused the skin cancers since the skin of industry workers comes into contact with uncured rubber containing many compounds--including aromatic amines.

N-Nitrosamines

Many, if not most, N-nitrosamines are known to be carcinogenic in laboratory animals. Humans are exposed to N-nitrosamines by a variety of routes (Fine, 1978)--food, tobacco, air, water, the workplace, and cosmetics. In addition, N-nitrosamines form through *in vivo* nitrosation.

Despite the known carcinogenicity of N-nitrosamines in animals and the known exposure of humans, there are no data indicating similar carcinogenicity in humans (International Agency for Research on Cancer, 1978a,b). Nor do data suggest that N-nitrosamines are *not* carcinogenic in humans. This lack of epidemiologic data on the health effects of N-nitrosamines in humans is due to the difficulty in identifying populations with known exposures to high levels of these substances. Exposure, when it occurs, is at relatively low levels. It is likely that, if adverse effects do occur, their rate is quite low. Furthermore, it may not be possible to separate the adverse effects of N-nitrosamines from those of other substances.

Two human health situations exist in which N-nitrosamines are suspected of being related to cancer. In Colombia, high rates of stomach cancer have been observed in an area where there arehigh nitrate concentrations (110mg/liter) in well water, together with a high rate of nitrate excretion by inhabitants (Cuello *et al.*, 1976). Because of this association, it has been hypothesized that *in vivo* nitrosation increases the incidence of stomach cancer (Correa *et al.*, 1975; Tannenbaum *et al.*, 1977). In the Peoples Republic of China, high rates of esophageal cancer occur both in humans and in poultry in an area where relatively high levels of nitrosamines and nitrites are present in food (Co-ordinating Group for Research on the Etiology of Esophageal Cancer in North China, 1974).

Nitrosamines have also been detected in alcoholic beverages (Goff and Fine, 1979; Walker *et al.*, 1979). It is generally accepted that alcohol is causally related to cancers of the pharynx, larynx, esophagus, and liver, primarily among heavy consumers of alcoholic beverages (Robinette *et al.*, 1979; Rothman, 1975; Schottenfeld, 1979; Williams and Horm, 1977). Breslow and Enstrom (1974), have reported a positive correlation between mortality rates for rectal cancer and heavy consumptions of alcohol as evidenced by tax receipts for the purchase of alcoholic beverages.

The specific agent in alcoholic beverages that is responsible for the increased cancer incidence is unknown, but the possibility that nitrosamines might lead to the increased rate cannot be ruled out. Two papers partially evaluated this possibility among Irish and Danish brewery workers (Dean *et al.*, 1979; Jensen, 1979). Both groups of workers receive free beer as part of their compensation.

At the time of those studies, the Irish beer contained low levels of nitrosamines; the Danish beer contained more (Goff and Fine, 1979; D.F. Fine, Personal communication).

Comparative data from these two groups of brewery workers are available only for cancers of the gastrointestinal tract. As seen in Table 3-1, an increased incidence of rectal cancer was found only among the Irish workers, who presumably consumed less nitrosamines than did the Danish workers; among Danish workers, only esophageal cancer appears in markedly increased incidence relative to the Irish workers. Inasmuch as esophageal cancer has repeatedly been found more frequently among alcoholics, the deficit among the Irish brewery workers seems to be the more atypical finding.

At present, it is not known whether nitrosamines have caused any cancer in humans. Because of the ubiquitous but low-level occurrence of these substances, it will be difficult to obtain epidemiologic evidence regarding the postulated association. However, as with all chemicals, it is prudent to lessen the potential for human exposure.

TABLE 3-1

Observed Gastrointestinal Cancers and Observed/Expected Ratios for Selected Cancers Among Irish and Danish Brewery Workers[a]

Cancer Site	Observed[b]		Obs./Exp. Ratio[c]	
	Irish	Danish	Irish	Danish
Esophagus	10	41	0.63	2.09
Stomach	40	92	0.94	0.88
Large intestine	32	87	1.17	1.07
Rectum	32	85	1.76	1.02
Liver	7	29	1.27	1.51
Pancreas	17	44	1.21	1.09

---

[a] Data abstracted from Dean *et al*., 1979 and Jensen, 1979.

[b] Irish numbers are deaths from cancer; Danish numbers are incidence rates of cancer.

[c] Expected numbers based on Dublin County Borough death rates (Irish) and from Danish national cancer morbidity rates.

REFERENCES

Breslow, N.E., and J.E. Enstrom. 1974. Geographic correlations between cancer mortality rates and alcohol-tobacco consumption in the United States. J. Natl. Cancer Inst. 53:631-639.

Co-ordinating Group for Research on the Etiology of Esophageal Cancer in North China. 1975. The epidemiology of esophageal cancer in North China and preliminary results in the investigation of its etiological factors. Sci. Sin. 18:131-148.

Correa, P., W. Haenszel, C. Cuello, S. Tannenbaum, and M. Archer. 1975. A model for gastric cancer epidemiology. Lancet 2:58-60.

Cuello, C., P. Correa, W. Haenszel, G. Gordillo, C. Brown, M. Archer, and S. Tannenbaum. 1976. Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. J. Natl. Cancer Inst. 57:1015-1020.

Dean, G., R. MacLennan, H. McLoughlin, and E. Shelley. 1979. Causes of death of blue-collar workers at a Dublin brewery, 1954-73. Br. J. Cancer 40:581-589.

Fine, D.H. 1978. An assessment of human exposure to N-nitroso compounds. Pp. 267-278 in E.A. Walker, M. Castegnaro, L. Griciute, and R.E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon.

Goff, E.U., and D.H. Fine. 1979. Analysis of volatile N-nitrosamines in alcoholic beverages. Food Cosmet. Toxicol. 17:569-573.

Hayhurst, E.R., and G.M. Kober. 1924. Poisonings in the rubber industry. Pp. 535-545 in G.M. Kober, and E.R. Hayhurst, eds. Industrial Health. P. Blakiston's Son & Co., Philadelphia.

International Agency for Research on Cancer. 1978a. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Volume 16. Some Aromatic Amines and Related Nitro Compounds--Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals. International Agency for Research on Cancer, Lyon. 400 pp.

International Agency for Research on Cancer. 1978b. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 17. Some N-Nitroso Compounds. International Agency for Research on Cancer, Lyon. 365 pp.

International Minerals & Chemical Corporation. 1979. 2-NP Mortality Epidemiology Study of the Sterlington, La. Employees 1-1-46 through 6-30-77, by M.E. Miller and G.W. Temple. International Minerals & Chemical Corporation, Mundelein, Ill. [44] pp.

Jensen, O.M. 1979. Cancer morbidity and causes of death among Danish brewery workers. Int. J. Cancer 23:454-463.

Monson, R.R., and L.J. Fine. 1978. Cancer mortality and morbidity among rubber workers. J. Natl. Cancer Inst. 61:1047-1053.

National Institute for Occupational Safety and Health. 1976. Suspected Carcinogens, 2nd Edition. A Subfile of the NIOSH Registry of Toxic Effects of Chemical Substances. H.E. Christensen and E.J. Fairchild, eds. HEW Publication No. (NIOSH) 77-149. U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Cincinnati. 251 pp.

Robinette, C.D., Z. Hrubec, and J.F. Fraumeni, Jr. 1979. Chronic alcoholism and subsequent mortality in World War II veterans. Amer. J. Epidemiol. 109:687-700.

Rothman, K.H. 1975. Alcohol. Pp. 139-150 in J.F. Fraumeni, Jr., ed. Persons at High Risk of Cancer; An Approach to Cancer Etiology and Control. Academic Press, New York.

Schottenfeld, D. 1979. Alcohol as a co-factor in the etiology of cancer. Cancer 43:1962-1966.

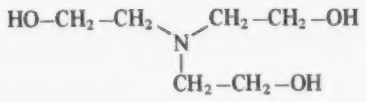
Tannenbaum, S.R., M.C. Archer, J.S. Wishnok, P. Correa, C. Cuello, and W. Haenszel. 1976. Nitrate and the etiology of gastric cancer. Pp. 1609-1625 in H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds. Origins of Human Cancer. Book C. Human Risk Assessment. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Walker, E.A., M. Castegnaro, L. Garren, G. Toussaint, and B. Kowalski. 1979. Intake of volatile nitrosamines from consumption of alcohol. J. Natl. Cancer Inst. 63:947-951.

Williams, R.R., and J.W. Horm. 1977. Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: Interview study from the Third National Cancer Survey. J. Natl. Cancer Inst. 58:525-547.

Chpater 4

TRIETHANOLAMINE



$$HO{-}CH_2{-}CH_2{-}\underset{\substack{|\\ CH_2-CH_2-OH}}{N}{-}CH_2{-}CH_2{-}OH$$

Triethanolamine (2,2',2"-nitrilotriethanol) is a pale yellow compound that solidifies at approximately room temperature. It has a melting point of 21.1°C and a boiling point of 335.4°C. Its vapor pressure is less than 0.01 mm Hg at 20°C. All three ethanolamines--mono-, di-, and triethanolamine--are produced by the reaction of ethylene oxide and aqueous ammonia at 50-100°C; the products are separated by distillation.

PRODUCTION

Four U.S. producers currently manufacture the three ethanolamines: Olin Corporation operates a plant in Brandenburg, Ky., with a capacity of 11,000 mt/year. Jefferson Chemical Co., Inc., a subsidiary of Texaco, Inc., increased the capacity of its Port Neches, Tex. plant from 36,000 to 68,000 mt/year in late 1979, and the Union Carbide Corporation plant in Seadrift, Tex. has a design capacity of 105,000 mt/year. Dow Chemical U.S.A. began operating a plant with 57,000-mt/year capacity in Plaquemine, La. in 1978. At that time, Dow also put a plant with 23,000-mt/year capacity at Freeport, Tex. on standby and converted another plant with a 23,000-mt/year capacity in Midland, Mich. to produce isopropanolamines. Allied Chemical Corporation has had a plant with 9,100-mt/year capacity on standby at Orange, Tex. since December 1973 (Stanford Research Institute International, 1976, 1977, 1978, 1979a,b).

U. S. production of triethanolamine, as reported to the U.S. International Trade Commission (1976-1979), was as follows: 1976, 47,575 mt; 1977, 47,611 mt; and 1978, 52,000 mt.

USES

Information on the consumption pattern for triethanolamine alone is not available; however, the consumption of all ethanolamines was reported for 1975 (Kirk-Othmer, 1978) and for 1979 (Chemical Marketing Reporter, 1979; U.S. International Trade Commission, 1979). These figures are shown in Table 4-1.

Triethanolamine is used principally as a chemical intermediate for anionic surfactants in the form of salts of rosin acids, fatty acids, alkyl benzene sulfonates, and alkyl sulfates. These in turn are used in household detergents, textile processing, cosmetics and toiletries, and metal-working compounds.

The two most widely used triethanolamines are the dedecyl sulfate (triethanolamine lauryl sulfate) and the salt of

TABLE 4-1

Ethanolamine Consumption Patterns, 1975[a] and 1979[b]

Uses	Quantity (10^3 mt)	Percentage of Total Consumed
1975		
Soaps and detergents	37.7	31.0%
Gas purification[c]	25.9	21.0
Cosmetics	10.5	8.6
Textile specialties	13.6	11.0
Agricultural products	4.5	3.6
Emulsion polishes	1.8	1.5
Construction	5.9	4.8
Metals	6.8	5.5
Chemical intermediates	10.9	8.9
Other	5.0	4.0
Total	122.6	100.0
1979		
Detergents	58.2	40.0%
Gas conditioning and petroleum use[c]	36.4	25.0
Other (including agriculture and construction)	29.1	20.0
Export	21.8	15.0
Total	145.5	100.0

---

[a] Kirk-Othmer, 1978.

[b] Chemical Marketing Reporter, 1979.

[c] The triethanolamine isomer is not used in gas purification and does not contribute to the consumption of ethanolamines in this application.

dodecylbenzenesulfonic acid (U.S. International Trade Commission, 1979). U.S. production of the dodecyl sulfate was 3,290 mt in 1978 and production of the salt of dodecylbenzenesulfonic acid in 1978 was 2,820 mt, according to the same source.

Other triethanolamine salts reported to be produced commercially in the United States in 1978 include the salts of (1) oleic acid, by Emkay Chemical Co., Elizabeth, N.J., and Diamond Shamrock Corporation, Harrison, N.J.; (2) stearic acid, by Glyco Chemicals, Inc., Williamsport, Pa., and Sybron Corporation, Wellford, S.C.; (3) undecylbenzenesulfonic acid, by Henkel Corporation, Hawthorne, Calif., and Witco Chemical Corporation, Houston, Tex.; (4) tallow acids, by Andrew Jergens Company, Saginaw, Mich.; and (5) mixed linear alcohols (sulfated), by Bofors Lakeway, Inc., Muskegon, Mich., and Henkel Corporation, Hawthorne, Calif. (Stanford Research Institute International, 1979). In addition to the use of triethanolamine to make the above surface-active agents, emulsions are commonly prepared *in situ*, with triethanolamine and fatty acids (Kirk-Othmer, 1978).

Other non-surface-active salts of triethanolamine commercially produced in the United States include (1) the phosphate (used as a corrosion inhibitor) and the monosulfate, both manufactured by Emkay Chemical Company, Elizabeth, N.J.; (2) the salicylate, produced by Norda, Inc., Boonton, N.J., and R.S.A. Corporation, Ardsley, N.Y.;and (3) the hydrochloride, produced by Eastman Kodak Company, Rochester, N.Y., and R.S.A. Corp., Ardsley, N.Y. (Stanford Research Institute International, 1979).

Other principal uses of triethanolamine are in agriculture and construction. The substance is used as an intermediate in the production of 2,4,5-trichlorophenoxyacetic acid, a triethanolamine salt, by Dow Chemical, U.S.A. at Midland, Mich. This chemical is registered for use as a herbicide by the U.S. Environmental Protection Agency (1974).

Triethanolamine is also used in the production of a triethanolamine-copper complex, marketed under the trade name A&V-70 Algaecide, by A&V Inc., Pewaukee, Wisc. This chemical is registered for use as an aquatic herbicide by the U.S. Environmental Protection Agency (1974).

In the construction industry, low levels of triethanolamine or its salts are added to cement clinkers to increase the efficiency of the grinding mill by reducing particle agglomeration (Chemical Marketing Reporter, 1979; Kirk-Othmer, 1978).

EXPOSURE

There is no information on the release of triethanolamine during either its production or its conversion to other products. Such emissions would be limited to the production sites mentioned above.

However, triethanolamine is used in the production of other chemicals at more than 60 sites, some in heavily populated areas, thus increasing the potential for exposure by atmospheric emissions.

The greatest potential for humans to be exposed to triethanolamine is probably through its food and cosmetics applications. The Food and Drug Administration (FDA) has approved the use of triethanolamine (1) in the formulation of adhesives for articles used in packaging or holding food; (2) in the formulation of resinous and polymeric coatings on food-contacting surfaces of items used in the processing, preparation, packaging, and holding of food; (3) in adjusting the pH during the manufacture of amino resins used as components of paper and paperboard in contact with aqueous and fatty foods; (4) as a component of coated or uncoated food-contacting surface of paper or paperboard used for dry food; (5) as a defoaming agent used in coatings of food-contacting surfaces or in the manufacture of paperboard or paper articles used in processing, preparation, packaging, and holding of food; (6) as a curing agent for polyurethane resins used on food-contacting surfaces; (7) as an activator in rubber articles intended for repeated use in food processing, packaging, and preparation; (8) as a component of textiles and textiles fibers used in articles involved in processing, preparation, and packaging of food; (9) in surface lubricants used in the manufacture of metallic articles that contact food; and (10) as a chemical used in washing or to assist in the lye peeling of fruits and vegetables (21 CFR 173). Neither the extent of contamination of foods by triethanolamine, nor even the degree of use in such applications, is known.

The use of triethanolamine in skin cleansers, lotions, and cosmetics is apparently widespread. The FDA lists triethanolamine as a component of shaving cream, shampoo, hair tonic, hair tint and dye, cleansing cream, foundation cream, hand cream, suntan lotion, lotion makeup base, rouge and blush makeup, mascara, eye shadow, cuticle removers, depilatories, and several other toiletries (Food and Drug Administration, 1980).

Because of its properties as a wetting agent, the pure form of triethanolamine may be added to cosmetics, especially to many creams and lotions, but it probably combines in situ with fatty acids to form triethanolamine fatty acid salts.

The Occupational Safety and Health Administration has not established standards for occupational exposures to triethanolamine. In the National Occupational Hazard Survey, which was conducted by the National Institute for Occupational Safety and Health (1977), exposures to triethanolamine derivatives (e.g., triethanolamine dodecylbenzenesulfonate, triethanolamine oleate) were reported in a number of industries. However, no exposures to triethanolamine itself were reported.

ANALYTIC METHODS

Because of the widespread uses of triethanolamine, it is often necessary to conduct analyses to determine its presence in waxes, oils, cosmetics, soaps, cutting fluids, and other organics. In all these analyses the first step is generally to remove the organic fraction by repeated extraction with methylene chloride. The residue is dried by evaporation on a steam bath and further cleaned up to remove acidic or alkaline fractions. The three hydroxyl groups render triethanolamine nonvolatile, and concentration is therefore readily carried out without loss by evaporation. If the sample can be purified sufficiently, final quantitation can generally be achieved by weighing the residue and ensuring its identity by infrared spectroscopy (Wisneski, 1977). More conventional procedures call for quantitation by gas chromatography, following volatilization by means of a suitable derivative.

Triethanolamine is readily derivatized at its three hydroxyl groups, thereby increasing the volatility so that it may be separated by gas chromatography. The following examples are some of the derivatives that may be used (Knapp, 1979).

o Methyl ether derivatives are prepared by reacting the triethanolamine with diazomethane in the presence of fluorboric acid.

o Acetyl derivatives are prepared by dissolving the compound in a 1:1 solution of acetic anhydride and pyridine. An alternative procedure involves the use of acetyl methanesulfonate in the presence of phosphorus pentoxide.

- *Pentafluorobenzoyl* derivatives are prepared by reaction of the parent amine with pentafluorobenzoyl chloride. The derivative is not only volatile, but can be analyzed using an electron capture detector on the gas chromotograph, thereby enhancing both selectivity and sensitivity.

- *Esters* are prepared by using the appropriate acid anhydride in the presence of toluenesulfonic acid.

- *Trimethylsilyl* derivatives are prepared by reacting the compound with hexamethyldisilazane (or any other suitable trimethylsilyl derivatizing agent).

Triethanolamine can be readily analyzed without derivatization by high pressure liquid chromatography.

HEALTH EFFECTS

Metabolism

No data were available on the metabolism of triethanolamine and little is known of the metabolism of its N-nitroso derivative NDElA, although it apparently requires metabolic activation to exert its mutagenic effects. When given to rats by gavage, 70% is excreted in urine as unchanged NDElA within 24 hours, possibly explaining the substance's rather weak carcinogenicity (Preussmann et al., 1978). This excretion level was independent of the administered dose range of 10-1000 mg/kg.

Because much human contact with NDElA is dermal, several studies have focused on this exposure route. Preussman and Spiegelhalder, (personal communication) reported that 70-80% of undiluted NDElA was absorbed through the shaved skin of rats. NDElA has also been shown to cross excised human skin (Bronaugh et al., in press) and the skin of a volunteer wearing a contaminated cosmetic (Edwards et al., 1979). An ancillary finding in the latter study was that the urinary excretion in the volunteer implied a half-life in humans of 12 hours.

Acute and Chronic Toxicity

In aquatic protozoa and invertebrates, the chronic and acute toxicity of triethanolamine was studied by determining the survival time following exposure (Apostol, 1975). Triethanolamine was less toxic than diethanolamine, which was less toxic than monoethanolamine. Chronic effects occurred at concentrations above 1 mg/liter; acute effects were observable at doses of more than 100 mg/liter. For mammals, all three compounds are weak toxins. No LD_{50} less than 700 mg/kg has been reported for any species or route of administration (National Institute for Occupational Safety and Health, 1977). Kostrodymova _et al_. (1976) showed that 13% triethanolamine solutions readily penetrated the skin of rat, causing changes in liver and central nervous system, which they claimed was indicated by increased levels of alanine aminotransferase and a decreased level of cholinesterase in blood. Topically applied triethanolamine did not exert carcinogenic or cocarcinogenic (with 3-methylcholanthrene) activity in mouse (Kostrodymova _et al_., 1976). When administered with synthenol DS-10 (a detergent used in the USSR), the substance inhibited the cocarcinogenic properties of the detergent.

Triethanolamine has been implicated as a source of occupational disorders in workers handling cutting fluids and other mixtures that contain the chemical. Calas _et al_. (1978) found evidence that triethanolamine was a sensitizer for contact allergens. Occupational dermatoses due to triethanolamine in cutting fluids (Selisskii _et al_., 1978) and in textile and finishing plant workers (Venediktova and Gudina, 1976) have also been reported.

Carcinogenicity

Hoshino and Tanooka (1978) recently reported that triethanolamine produced malignant tumors in mice. Male and female ICR/JCL mice were fed a diet containing 0.03% or 0.3% triethanolamine for their lifetimes. In the females, there was a significant increase in the total number of malignant tumors in both the 0.03% and 0.3% groups as compared to the controls (*P* < 0.01). There was a slight, but not statistically significant dose response noted. Predominant tumor types were thymic and nonthymic lymphomas in the females; both sexes also had tumors in several other sites. No hepatomas were observed. Because the purity of the material first fed to the animals was not thoroughly established, and because the nature of any products formed as a result of mixing the triethanolamine with the diet was not studied, the identity of the carcinogenic material remains in doubt. No other references were found regarding the possible carcinogenicity of triethanolamine.

NDElA, has been shown to be present as an impurity in triethanolamine (Fine, unpublished data, 1978), in many products that contain triethanolamine, such as cosmetics (Fan *et al*., 1977a), and in cutting fluids (Fan *et al*., 1977b; Zingmark and Rappe, 1977).

The carcinogenicity of NDElA has been studied in both rat and hamster. A group of 20 BD rats was given NDElA in drinking water at concentrations equivalent to 600-1,000 mg/kg/day, the total dose was 150-300 g/kg. All 20 animals developed hepatocellular carcinomas between 242 and 325 days after the start of treatment; four rats also had renal adenomas (Druckrey *et al*., 1967). In Syrian golden hamsters, two groups of 15 males and 15 females received either seven twice-weekly subcutaneous injections each of 2,260 mg/kg NDElA (one-fifth of the LD_{50}) or 27 injections of 565 mg/kg (one-twentieth of the LD_{50}) over 45 weeks. For the latter group, several injection-free intervals of 1-2 weeks were required because of local necrosis at the injection site. Total doses were approximately 15 g/kg. All surviving animals were killed at 78 weeks. In the first group, 28 of 30 animals were still alive at the appearance of the first tumor (33 weeks); 20 developed tumors--including 10 adenocarcinomas of the nasal cavity, 8 papillary tumors of the trachea, and 3 hepatocellular adenomas. In the second group, 27 of 30 animals survived 33 weeks; 19 developed tumors--including 12 adenocarcinomas of the nasal cavity, 8 papillary tumors of the trachea, and 3 fibrosarcomas at the injection site. Of 27 effective controls, 3 animals developed 1 thyroid carcinoma, 1 hemangioendothelioma of the spleen, and 2 adenomas of the adrenal gland (Hilfrich *et al*., 1978).

Mutagenicity of Triethanolamine

Triethanolamine has not been well studied; however, the studies that have been performed show no evidence of mutagenic activity in bacteria.

Bacteria. Hoshino and Tanooka (1978) exposed *Bacillus subtilis* TKJ5211 (uvr) to analytical-grade triethanolamine in the presence and absence of sodium nitrate. An increase in the frequency of his^+ revertants was seen when triethanolamine was mixed with an equal amount of sodium nitrite and allowed to react for 8 hours. This activity was greater than that observed with sodium nitrite alone. In the presence of rat liver S-9, the mutagenic activity was abolished. Triethanolamine alone or with rat liver S-9 was not mutagenic. The same triethanolamine-sodium nitrite mixture, following autoclaving, was more toxic to *B. subtilis* wild type strain than to the corresponding DNA repair-deficient strains. However, these strains were not isogenic. The mutagenic activity observed only in the absence of S-9 argues against NDElA as the mutagen; however, the study did not test NDElA, so the possibility that it was the mutagen formed cannot be excluded.

Triethanolamine was also tested for mutagenicity in *Salmonella* through the Environmental Mutagenesis Test Development Program of the National Institute for Environmental Health Sciences (NIEHS). The test system used was a preincubation modification of the *Salmonella*/microsome test using Aroclor-induced rat and Syrian hamster liver S-9 and strains TA98, TA100, TA1535, and TA1537. No mutagenic activity was observed at dosage levels of up to 3.3 mg/plate (W. Speck, personal communication).

Plants. Triethanolamine, at a concentration of 0.125 mol/liter, did not induce multipolar mitoses in *Allium cepa* cells after 4 hours of treatment (Barthelmess and Elkabarity, 1962).

Mutagenicity of N-Nitrosodiethanolamine

The results obtained with NDElA are summarized in Table 4-2. The only available mutagenicity data on NDElA were obtained from studies using *Salmonella*, *Escherichia coli*, and *B. subtilis*. The reported results are mixed, showing both positive and negative results in *Salmonella*. Some authors report that liver S-9 is required; others find no requirement for liver S-9.

TABLE 4-2
Summary of Mutagenicity Tests with N-Nitrosodiethanolamine (NDElA)

Species/Strain	Results	References
S. typhimurium TA1535	Negative	Rao *et al.*, 1979
S. typhimurium GA46, TA100	Positive[a,b]	McMahon *et al.*, 1979
S. typhimurium TA1535, C3076 TA1537, D3052, TA1538, TA98	Negative[a]	McMahon *et al.*, 1979
S. typhimurium TA100, TA1535	Positive[c]	Hesbert *et al.*, 1979
S. typhimurium TA98, TA1538	Negative	Hesbert *et al.*, 1979
E. coli WP2, WP2uvrA	Positive[a,b]	McMahon *et al.*, 1979

---

[a] Streak test protocol.
[b] With rat liver S-9 only.
[c] Both with and without mouse liver S-9.

Bacteria. Rao *et al*. (1979) tested NDE1A in *Salmonella* TA1535 at concentrations up to 2,000 μg per plate in the standard plate test and up to 2,000 μg/ml in the preincubation modification test. All tests were run both with and without phenobarbital-induced rat liver S-9. No positive responses were observed.

NDE1A was reported to be mutagenic for both TA1535 and TA100 (Hesbert *et al*., 1979), both with and without S-9 activation, on a standard plate test at concentrations up to 10 mg/plate. A positive response was observed at 2 mg/plate with TA1535, which appears to contradict the negative report from Rao *et al*. (1979). In another study, McMahon *et al*. (1979) used a gradient plate test. In this procedure, a concentration gradient (ranging from 1 to 10 mg/ml in the agar) was formed in a Petri dish, and the microbial tester strain was streaked across the gradient. NDE1A was tested against a series of *Salmonella* and *E*. *coli* tester strains, both with and without Aroclor-induced rat liver S-9. NDE1A produced positive results only with S-9 in *Salmonella* strains G46 and TA100 and *E*. *coli* strains WP2 and WP2uvrA. No mutagenicity was observed either in TA1535 or any of the *Salmonella* frameshift-specific strains used. This result appears to be in agreement with the negative results produced by Rao *et al*. (1979). However, strains G46, TA100, and TA1535 all measure reversion at the same locus. G-46 is just *his*$^-$; TA1535 is strain G46 with an additional *rfa* mutation and *uvrB* *bio* *gal* deletion. TA100 is TA1535 containing the plasmid pKM101, which enhances its sensitivity and decreases its specificity. E. coli WP2 and WP2A detect base-pair, substitution-inducing substances. There is no obvious explanation for the negative results produced by TA1535 and the positive results produced by G46 and TA100.

In a different study (Hoshino and Tanooka, 1978), NDElA was not studied directly, but the mixture of triethanolamine and sodium nitrite at pH 3.5 (which is supposed to produce NDElA) was mutagenic for a B. subtilis mutant along with a series of isogenic repair-deficient strains. A mutagen formed during the triethanol-sodium nitrite reaction was active without S-9. It lost its activity in the presence of Aroclor-induced rat liver S-9. Triethanolamine alone was not mutagenic; sodium nitrite alone showed some mutagenicity, but significantly less than that of the mixture. The repair-deficient strains were more sensitive to killing and mutagenesis than was the wild-type strain. The investigation concluded that the mutagen formed in the reaction mixture was not NDElA because the mutagen was active only in the absence of S-9. However, since no NDElA control was run, the conclusion was not substantiated.

Teratogenicity

No data were available to evaluate the potential teratogenicity or reproductive toxicity of triethanolamine.

CONCLUSIONS

Triethanolamine does not induce point mutation in bacteria, and its ability to induce point or chromosomal mutations in eukaryotic cells has not been tested. The chemical is also of interest because of its ability to be nitrosated to form NDElA. *In vitro* and *in vivo* mutagenicity studies of NDElA formation, coupled with analyses of NDElA yield, must be performed.

From the results described here, it is obvious that more studies of NDElA will have to be performed to resolve the apparent discrepancies. There is no agreement as to the strains that are mutated or their requirement for liver S-9 fraction. Certainly, NDElA is a mutagen for *Salmonella*, but the conditions under which it is mutagenic still have to be determined. When mutagenicity was observed, it usually occurred after exposure to high doses, bringing into question the purity of the NDElA tested and possible impurities present in the sample. No studies have been performed to evaluate the induction of chromosomal mutation. Therefore, in addition to further testing with microbial systems, studies should also be performed *in vitro* in mammalian systems.

Studies should be conducted to determine human exposure levels to both triethanolamine and to NDElA and to further assess their potential noncarcinogenic toxicity, teratogenicity and effects on reproduction.

REFERENCES

Production, Uses, Exposure

Chemical Marketing Reporter. 1979. Chemical profiles: Ethanolamines. Chem. Mark. Rep. 215(15):9, April 9. Schnell Publishing Co., New York.

Code of Federal Regulations. 1980. Title 21, Part 173. Secondary direct food additives permitted in food for human consumption. Office of the Federal Register, National Archives and Records Service, General Services Administration, Washington, D.C.

Food and Drug Administration. 1980. Information received under 21 CFR Part 720 by the Division of Cosmetic Technology, Food and Drug Administration, Washington, D.C.

Kirk-Othmer Encyclopedia of Chemical Technology. 1978. Third Edition, Vol 2. Martin Grayson, exec. ed. and David Eckroth, assoc. ed. A Wiley-Interscience Publication, John Wiley & Sons, New York. 1,036 pp.

Stanford Research Institute, Chemical Information Services. 1976. 1976 Directory of Chemical Producers: United States of America. Stanford Research Institute, Menlo Park, Calif. 1,039 pp.

Stanford Research Institute, Chemical Information Services. 1977. 1977 Directory of Chemical Producers: United States of America. Stanford Research Institute, Menlo Park, Calif. 1,060 pp.

Stanford Research Institute International, 1978. 1978 Directory of Chemical Producers: United States of America. Stanford Research Institute International, Menlo Park, Calif. 1,127 pp.

Stanford Research Institute International, 1979a. 1979 Directory of Chemical Producers: Western Europe. 2 volumes. Stanford Research Institute International, Menlo Park, Calif. 2,100 pp.

Stanford Research Institute International, 1979b. 1979 Directory of Chemical Producers: United States of America. Stanford Research Institute International, Menlo Park, Calif. 1,122 pp.

U.S. Environmental Protection Agency. 1974. EPA Compendium of Registered Pesticides, Volumes I-V. U. S. Environmental Protection Agency, Washington, D.C.

U. S. International Trade Commission. 1976. Synthetic Organic Chemicals, U. S. Production and Sales, 1974. USITC Publication 776. U.S. International Trade Commission, Washington, D.C. 256 pp.

U. S. International Trade Commission. 1977a. Synthetic Organic Chemicals, U. S. Production and Sales, 1975. USITC Publication 804. U.S. International Trade Commission, Washington, D.C. 246 pp.

U. S. International Trade Commission. 1977b. Synthetic Organic Chemicals, United States Production and Sales, 1976. USITC Publication 833. U.S. International Trade Commission, Washington, D.C. 357 pp.

U. S. International Trade Commission. 1978. Synthetic Organic Chemicals, U. S. Production and Sales, 1977. USITC Publication 920. U.S. International Trade Commission, Washington, D.C. 417 pp.

U. S. International Trade Commission. 1979. Synthetic Organic Chemicals, U. S. Production and Sales, 1978. USITC Publication 1001. U.S. International Trade Commission, Washington, D.C. 369 pp.

Analytic Methods

Knapp, D.R. 1979. Handbook of Analytical Derivatization Reactions, Wiley and Sons, New York , 741 pp.

Wisneski, H.H. 1977. Analysis of cold wave solutions. Pp. 78-82 in A.J. Senzel, ed. Newburger's Manual of Cosmetic Analysis. 2nd Edition. Association of Official Analytical Chemists, Inc., Washington, D.C.

Health Effects

Apostol, S. 1975. Ethanolamine toxicity to aquatic invertebrates. Stud. Cercet. Biol. 27:345-351. [Chem. Abs. 85:73051q, 1976]

Barthelmess, A., and A. Elkabarity. 1962. Chemically induced multipolar mitosis. III. Protoplasma 54:455-475. [Chem. Abs. 57:3891a, 1962]

Bronaugh, R.L., E.R. Congdon, and R.J. Scheuplein. in press. The effect of cosmetic vehicles on the penetration of N-nitrosodiethanolamine through excised human skin. J. Invest. Dermatol.

Calas, E., P.Y. Castelain, and A. Piriou. 1978. Epidemiologie des dermatoses de contact a marseille. Ann. Dermatol. Venerol. 105:345-347. (English abstract) [Cumul. Ind. Medicus 19:1640, 1978]

Druckrey, H., R. Preussmann, S. Ivankovic, and D. Schmaehl. 1967. Organotrope carcinogenic effects of 65 different N-nitroso compounds on BD-rats. Z. Krebsforsch. 69:103-201. [Chem. Abs. 67:32277v, 1967].

Edwards, G.S., M. Peng, D.H. Fine, B. Spiegelhalder, and J. Kann. 1979. Detection of N-nitrosodiethanolamine in human urine following application of a contaminated cosmetic. Toxicol. Lett. 4:217-222.

Fan, T.Y., U. Goff, L. Sing, D.H. Fine, G.P. Arsenault, and K. Biemann. 1977a. N-Nitrosodiethanolamine in cosmetics, lotions and Shampoos. Food cosmet. Toxicol. 15:423-430.

Fan, T.Y., J. Morrison, D.P. Rounbehler, R. Ross, D.H. Fine, W. Miles, and N.P. Sen. 1977b. N-Nitrosodiethanolamine in synthetic cutting fluids: A part-per-hundred impurity. Science 196:70-71.

Hesbert, A., M. Lomonnier, and C. Cavelier. 1979. Mutagenicity of nitrosodiethanolamine on Salmonella typhimurium. Mutat. Res. 68:207-210

Hilfrich, J., I. Schmeltz, and D. Hoffmann. 1978. Effects of N-nitrosodiethanolamine and 1,1-diethanolhydrazine in Syrian golden hamsters. Cancer Lett. 4:55-60.

Hoshino, H., and H. Tanooka. 1978. Carcinogenicity of triethanolamine in mice and its mutagenicity after reaction with sodium nitrite in bacteria. Cancer Res. 38:3918-3921.

Kostrodymova, G.M., V.M. Voronin, and N.N. Kostrodymov. 1976. The toxicity (in complex action) and the possibility of cancerogenic and cocancerogenic properties of tri-ethanolamines. Gig. Sanit., No. 3:20-25. [in Russian, English summary]

McMahon, R.E., J.C. Cline, and C.Z. Thompson. 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. Cancer Res. 39: 682-693.

National Institute for Occupational Safety and Health. 1977. Registry of Toxic Effects on Chemical Substances. 2 Vols. DHEW Publication Nos. (NIOSH) 78-104-A and B. U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Cincinnati. [GPO Monthly Catalog No. 1006:192, entry 17630, Sept. 1978]

Preussmann, R., G. Wurtele, G. Eisenbrand, and B. Spiegelhalder. 1978. Urinary excretion of N-nitrosodiethanolamine administered orally to rats. Cancer Lett. 4:207-209.

Rao, T.K., J.A. Young, W. Lijinsky, and J.L. Epler. 1979. Mutagenicity of aliphatic nitrosamines in *Salmonella typhimurium*. Mutat. Res. 66:1-7.

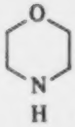
Selisskii, G.D., A.S. Obukhova, A. Anton'ev, B.A. Somov, M.V. Shaparenko, and L.V. Alchangyan. 1978. Prophylaxis of occupational dermatoses caused by inhibitors of atmospheric metal corrosion. Vestn. Dermatol. Venerol., No. 9:36-39. [Chem. Abs. 90:173844q, 1979]

Venediktova, K.P., and R.V. Gudina. 1976. Clinical-immunological characteristics of allergic dermatitis and eczema in textile workers. Vest. Dermatol. Venerol., No. 10:32-37. Chem. Abs. 87:28220s, 1977]

Zingmark, D.A., and C. Rappe. 1977. On the formation of N-nitrosodiethanolamine in a grinding fluid concentrate after storage.Ambio 6:237-238.

Chapter 5

MORPHOLINE



Morpholine (tetrahydro-2*H*-1,4-oxazine) is an oxygen-containing cyclic amine. It is a colorless, hygroscopic liquid with a melting point of 4.9°C and a boiling point of 128.9°C. It is completely miscible with water in all proportions. Its vapor pressure is 8.0 mm Hg at 20°C.

Morpholine is produced by reacting diethylene glycol, ammonia, and a small amount of hydrogen over a hydrogenation catalyst at 150-400°C and 30-400 atm. The morpholine product is recovered by fractional distillation. Among the byproducts are 2-(2-aminoethoxy)ethanol and *N*-alkylmorpholines.

PRODUCTION

Currently, the major U.S. producer of morpholine is the Jefferson Chemical Co., a subsidiary of Texaco, Inc. Its Port Neches, Tex. plant has a capacity of 12,700 mt/year (Stanford Research Institute International, 1975). Jefferson also has a plant with a capacity of 6,800 mt/year at Conroe, Tex., which has been on standby status since 1976 (Anonymous, 1980). In addition, Dow

Chemical Co. has had a plant with a capacity of 2,300 mt/year on standby at Midland, Mich. since 1972.

Total morpholine production has been stable at about 11,000 mt/year since 1974. In addition to this domestic production by Jefferson Chemical, BASF Wyandotte imports an estimated 900 mt of morpholine per year (Anonymous, 1980). In October 1979, BASF Wyandotte announced that it had begun engineering studies for a plant to produce 8,200 mt/year at a site in Geismar, La. Another potential producer is Air Products and Chemicals Co., which is considering building a plant of unspecified capacity at Pace, Fla. The company will use a new, low-pressure process (Anonymous, 1980).

USES

Morpholine has a multiplicity of uses. The largest single use is as an intermediate in the production of rubber chemicals, principally delayed-action rubber accelerators, stabilizers against heat-aging effects, and bloom inhibitors in butyl rubber vulcanization. Because of the similarity in vapor pressures of morpholine and water, and because of morpholine's neutralizing effects on carbonic acid, the chemical is also used extensively as a corrosion inhibitor in steam boiler systems (Kirk-Othmer, 1978).

Morpholine reacts with fatty acids to form soaps with excellent emulsifying properties. These products find use in household and automotive waxes and polishes. The morpholine salt of stearic acid is produced by Imoco-Gateway Corporation in Baltimore, Md.

Other miscellaneous uses of morpholine itself include formulations in cosmetic products. The chemical is used in eye shadow, eyeliner, and mascara, in percentages ranging from 0.1 to 5.0% (Food and Drug Administration, 1980). It is also used in the production of other derivatives. N-Morpholyl formamide, used for benzene, toluene, and xylene extraction, is produced by Fike Chemical Company, Nitro, W. Va. Morpholine hydroperiodide is used as a pharmaceutical and as a disinfectant.

According to the U.S. International Trade Commission (1978), commercially significant morpholine derivatives used as accelerators include 4-morpholinyl-2-benzothiazyl disulfide[N,2-morpholinothio)-benzothiazole], produced by Goodyear Tire and Rubber Co., Akron, Ohio; N-oxydiethylene-2-benzothiazolesulfenamide[2-(4-morpholinothio)benzothiazole], produced by American Cyanamid Co., Bound Brook, N.Y., The Goodyear Tire and Rubber Co., Akron, Ohio, Pennwalt Corp., Wyandotte, Mich., and B. F. Goodrich Co., Henry, Ill.; and N-(2,6-dimethylmorpholino)benzothiazolesulfenamide, produced until 1978 by Monsanto Company, Nitro, W. Va., and currently produced by Uniroyal Chemical Co., Naugatuck, Conn.; bis(morpholinothiocarbonyl) disulfide is produced by American Cyanamid Co., Bound Brook, N.Y.

N,N'-Dithiodimorpholine, produced by Monsanto Co., Nitro,

W. Va., and R.T. Vanderbilt Co., Inc., Bethel, Conn., is the only commercially significant morpholine derivative used to stabilize rubber against heat-aging effects. (U.S. International Trade Commission, 1978). Dimorpholine polysulfide is used as a bloom inhibitor in butyl rubber vulcanization.

4,4'-Bis[(4-analino-4-morpholino-1,3,5-triazin-2yl)amino]stilbene-2,2'-disulfonic acid, disodium salt, also known as C.I. Fluorescent Brightening Agent 260, is used as an optical brightener for cellulose and soaps and detergents.

Two short-chain alkyl morpholines, 4-ethylmorpholine and 4-methylmorpholine, are used as catalysts in the manufacture of polyurethanes (Kirk-Othmer, 1978). They are used with other catalysts to give a balanced foaming system for polyurethane foam production. These compounds are produced by Lonza, Inc., Mapleton, Ill.; Jefferson Chemical Co., Conroe, Tex.; and Union Carbide Corp., South Charleston, W. Va.

A number of long-chain alkyl morpholines are also produced. *N*-(Coconut oil alkyl)morpholine and *N*-n-dodecyl morpholine are produced by Lonza, Inc., in Mapleton, Ill. The compounds are believed to be used primarily as intermediates for the corresponding amine oxides, which are used as surfactants. Two other long-chain alkyl morpholines, *N*-*N*-hexadecylmorpholine and *N*-(soybean oil alkyl) morpholine are used as intermediates for the corresponding quaternary ammonium salts, *N*-ethyl-*N*-hexadecylmorpholinium ethyl sulfate and N-ethyl-N-(soybean oil alkyl) morpholinium ethyl sulfate, which are cationic surfactants. All four are produced by ICI America, Inc., in New Castle, Del.

6-Morpholino-4,4-diphenyl-3-heptanone hydrochloride (phenadoxone hydrochloride) is used as a pharmaceutical. Morpholine salts of acylated sulfonamides are used as bactericides. The morpholine salt of p-toluenesulfonic acid, used as a catalyst to produce epoxy resin coatings for articles that come into contact with food, is produced by American Bio-Synthetics Corp., Milwaukee, Wisc. Morpholine borane is produced by Alfa Products, Inc. in Denvers, Mass., a subsidiary of Thiokol Corporation.

The various uses of morpholine, based on total consumption of 11,000 mt/year, are summarized in Table 5-1.

EXPOSURE

Because of the numerous uses of morpholine, there are many potential routes of human exposure. Releases to the atmosphere occur primarily during the production of morpholine and its derivatives.

Atmospheric morpholine releases were estimated in a study conducted for the Environmental Protection Agency (EPA) by Science Applications, Inc. (1980). Emissions from morpholine production were based on analogy with emissions from ethylene oxide production

TABLE 5-1

Uses of Morpholine[a]

Uses	Amount (mt/yr)	Percentage of Total
Used		
Rubber Chemicals	3,600	33
Corrosion inhibitors	2,700	25
Optical brighteners	1,100	10
Alkyl morpholines	1,100	10
Waxes and polishes	900	8
Exports	800	7
Miscellaneous uses	800	7

---

[a] From Chemical Marketing Reporter, 1974, with permission.

in the absence of any actual emissions data. Total production-related emissions were estimated to be 6,200 kg/year for a production level of 11,000 mt/year. The exposed population would include only those persons living in Port Neches, Tex. (population 11,000) and the surrounding area.

In the same study (Science Applications, Inc., 1980), the emissions of morpholine from the various uses listed in Table 5-1 were also estimated. For the production of rubber chemicals and optical brighteners, an emission factor of 0.001 kg per kilogram of morpholine used was assumed. The emissions were assumed to be equally distributed among 96 (unspecified) sites where rubber accelerators are produced, and 128 (unspecified) sites where optical brighteners are produced. The total annual emissions from these sources were estimated at 5,100 kg/year. Miscellaneous morpholine uses were assumed to have the same emissions factor, for a total emission rate of 900 kg/year, distributed in proportion to the U.S. population.

The study assumed that all morpholine used as a corrosion inhibitor (2,700 mt/year) and in waxes and polishes (1,100 mt/year) would be emitted to the atmosphere. However, such an assumption appears to result in a substantial overestimate of atmospheric emissions. The use of morpholine as a boiler additive to inhibit corrosion involves adding the chemical to the boiler feedwater, and the boiler water-stream system is a closed system involving little if any release to the atmosphere. However, a portion of the boiler water is occasionally discharged (blown down) to lower the buildup of salts and chemicals in the system. Therefore, the ultimate fate of morpholine in the system is in the boiler blowdown stream.

The discharge of morpholine in blowdown streams from industrial and utility boilers is a potential source of contamination of rivers and streams. In a study of toxic chemicals in power plant discharges (McCain and Peck, 1976), the concentration of morpholine was measured in three Hawaiian power plants known to use morpholine as a boiler feedwater additive. The morpholine concentrations detected in various power plant discharge streams ranged from no detectable amount to a maximum of 0.008 ppm. If these figures are typical, then the potential for human exposure, given the further diluting effect of the receiving water bodies, appears to be very small.

In soaps, polishes, and waxes, morpholine is typically used in the form of salts of fatty acids. Therefore, it is unlikely that any pure morpholine is released to the atmosphere through evaporation.

The Food and Drug Administration (FDA) has approved the use of morpholine and its derivatives in various applications, including protective coatings applied to fruits and vegetables, boiler water additives for steam generation used for food preparation, adhesives for food packaging, and defoaming agents used in paper and paperboard manufacture (21 CFR 172). Possibly as a result of such uses, morpholine has been found in a number of food products (Singer and Lijinsky, 1976). The results of the analyses are summarized in Table 5-2.

TABLE 5-2
Concentrations of Morpholine in Various Food Products[a]

Substance	Morpholine Concentration (ppm)
Canned tuna	≤ 0.7
Frozen ocean perch	10.0
Frozen cod	<0.3
Spotted trout	7.0
Small mouth bass	≤ 0.8
Salmon	1.2
Baked ham	0.5
Frankfurters	0.4
Evaporated milk	0.2
Coffee	1.0
Tea	<0.1
Canned beer	0.4
Bottled beer	≤ 0.2
Wine	≤ 0.7

---

[a] From Singer and Lijinsky, 1976, with permission.

The ubiquitous occurrence of morpholine in the diverse types of food products examined leads to the suspicion that morpholine is present in parts-per-million quantities in many different types of foodstuffs and that this presence constitutes a rather widespread route of exposure.

Another route of human exposure derives from the widespread use of morpholine in cosmetics, primarily in eyeliner and mascara, although a large percentage of commercial formulations contain morpholine. The exposure route is inhalational (as well as) dermal, because of the relatively high vapor pressure of morpholine.

Finally, occupational exposures may be significant. A National Occupational Hazard Survey conducted by the National Institute for Occupational Safety and Health (NIOSH) detected worker exposure to morpholine in 283 different industries (as specified by the Standard Industrial Classification four-digit code). Worker exposure to morpholine is currently regulated by the Occupational Safety and Health Administration (OSHA). The OSHA standard for morpholine in air is a time-weighted average of 20 ppm for 8 hours (Occupational Safety and Health Administration, 1980).

ANALYTIC METHODS

Singer and Lijinsky's (1976a,b) procedure for foodstuffs such as fish, beer, wine, tea, coffee, milk, water, frankfurters, tobacco, and tobacco condensates is based on conversion to *p*-toluenesulfonamide, and then gas chromatography-mass spectrometry (GC-MS) of the derivatized amine. The first step in the analysis procedure is acidification to pH 1, mixing in a blender with water, and then adjustment to pH 10 with sodium and barium hydroxide. The alkaline mixture is then steam distilled into acid, extracted with ether to remove neutral compounds, concentrated to 3 ml, and then derivatized by refluxing with an alkaline solution of *p*-toluenesulfonyl chloride. The derivative is further purified and then analyzed by GC-MS; carbon-14-labelled amine is used as an internal recovery standard. The sensitivity of the method is approximately 0.3 ppm (by weight). Morpholine is found to be present in most samples analyzed.

Tombropoulos (1979) has developed a much simplified procedure, in which the morpholine is analyzed directly by GC using a Chromosorb 103 column and a flame ionization detector. Better specificity is obtained if a nitrogen-selective GC detector, such as an alkaline flame ionization detector or a Hall detector, is used. The method has been applied to blood, urine, and other biological samples.

Karweik and Meyers (1979) have developed a method that utilizes diakyldithiocarbamate copper complex, which is formed from the reaction of the aliphatic amine with carbon disulfide in the presence of ammonia. The copper bis(dithiocarbamate) complex is then extracted with chloroform and its concentration determined spectrophotometrically at 434 nm.

Airborne morpholine in industrial atmospheres where morpholine-based cutting fluids are used has been monitored by trapping the morpholine in a 0.025% aqueous solution of methyl orange, followed by colorimetry (Burenko _et_ _al_., 1977). Fajen _et_ _al_. (1979) determined airborne morpholine in chemical and tire factories by trapping in 1-N potassium hydroxide solution, acidifying to pH 3, nitrosating with sodium nitrite, and then measuring the increase in the _N_-nitrosomorpholine concentrations.

HEALTH EFFECTS

Acute Toxicity

The LC_{50} of morpholine (continuous exposure) has been reported to be 2,250 ppm for male and female rats and 1,450 ppm and 1,900 ppm for male and female mice (Lam and Van Stee, 1978).

Shea (1939) exposed rats and guinea pigs at 18,000 ppm (63,000 mg/m^3) continuously for 8 hours or intermittently for up to 42 hours. Some deaths resulted; irritation of the eyes and nose, hemorrhaging in lungs, and congestion of liver and kidneys were also reported. In other studies, rats exposed to 450 ppm of morpholine for 6 hours/day, 5 days/week for 8 weeks exhibited a decrease in food consumption and body weight gain as well as an increased organ-to-body-weight ratio for lungs and kidneys. Changes to sensory areas such as the eyes and nose were also noted (Lam and Van Stee, 1978). Shea (1939) reported that, after exposing himself to morpholine at 12,000 ppm (42,000 mg/m^3) for 1.0-1.5 minutes, he experienced irritation of the nose and coughing. In a related study (Pennsylvania Department of Health, 1967-1969), humans exposed to N-ethylmorpholine for 2-3 minutes at 50-100 ppm (175-350 mg/m^3) experienced upper respiratory tract irritations and complained of an ammonia-like odor. At 25 ppm (87.5 mg/m^3), the only finding was detection of a noticeable odor. In a survey of a worksite where N-ethylmorpholine was being used, workers described a visual "halo" effect during a 2-hour exposure to airborne concentrations of 6-22 ppm (21-77 mg/m^3). This effect lasted for up to 2 hours after exposure ended. As a result of these findings, the Pennsylvania Department of Health recommended a short-term exposure limit (15 minutes) of 20 ppm for both morpholine and N-ethylmorpholine.

The American Conference of Governmental Industrial Hygienists (1974) reported that the primary effects of exposure to airborne morpholine are nasal and bronchial irritation and liver damage. They compared morpholine's action to that of ammonia but, because of greater potential for systemic effects, suggested that a somewhat lower environmental limit for morpholine was appropriate. However, the threshold-limit value/time-weighted average (TLV-TWA) concentration limit of 20 ppm (70 mg/m^3) was still recommended on the basis of protection against irritation and damage to the eyes.

According to Ivanov and Germanova (1973), men exposed to morpholine at a concentration of 16 mg/m^3 complained of irritation after exposure of only 1 minute. The authors thus characterized that concentration as the threshold for irritating action in humans. Results from other short-term inhalation exposures to morpholine are shown in Table 5-3.

A series of Russian studies has resulted in reports of other effects after exposure to airborne levels of morpholine considerably lower than those previously mentioned. In one experiment (Ivanov *et al.*, 1973), rats were exposed for 4 hours to morpholine at 260, 40, or 3 mg/m^3. Morpholine toxicity was evaluated by measuring respiratory rate, lung weight, and uptake of stain by lung tissue.

Table 5-3
Effects of Short-Term Inhalation Exposure to Morpholine

Species	Concentration ppm	Concentration mg/m^3	Time (hours)	Effects	References
Rat	8,497	29,740	8	1/6 deaths	International Labour Office, 1972
Rat	8,000	28,000	8	No deaths	Smyth *et al.*, 1954
Rat	6,734	23,569	4	Signs of irritation	International Labour Office, 1972
Rat	6,285	22,000	1	Lacrimation, rhinitis, inactivity	Industrial Bio-Test Laboratories, Inc., 1970
Mouse	1,391	4,869	?	LC_{50}	Zaeva *et al.*, 1968

To measure the degree of lung staining, a 1% solution of neutral red stain was injected into the tail vein after the animals were exposed to morpholine. The rats were killed 1 and 10 minutes later, and then ; 0.5-g sections of the lungs were removed to extract the stain. Measurements at 1 and 10 minutes corresponded to points of maximum accumulation and secretion of the dye, based on observations in preliminary experiments. In undamaged cells the dye was taken up, stored as a granule, and then removed. Damaged cells lost the ability to collect the stain in a granule; instead, the nucleus and cytoplasm became stained by diffusion. At 260 mg/m^3, the investigators observed an increase in respiratory rate, but no effect on lung weight. The exposed rats also retained a greater amount of stain than did controls after 1 and 10 minutes. Rats exposed to a morpholine dosage of 40 mg/m^3 had eliminated less stain from lung tissue after 10 minutes than had controls. No changes in respiratory rate or lung weight were observed at dosages of 40 or 3 mg/m^3. The investigators reported the test for stain removal from lung tissue to be the most sensitive indicator of irritation. Because 40 mg/m^3 was the lowest tested airborne concentration that caused a change in removal of stain from lungs, that amount was characterized as the threshold limit for irritation in rats.

Grodetskaya and Karamzina (1973) evaluated thyroid function as another indicator of toxicity. They measured the uptake of iodine-131. Male rats were exposed to 80 mg/m^3 of morpholine 4 hours/day for 2, 4, or 8 days and then administered iodine -131.

Thyroid gland uptake was measured over 48 hours. Exposed rats accumulated a larger amount of iodine-131 than did controls, indicating increased thyroid gland activity. Microscopic examination of the thyroid gland showed hypersecretion by thyroid cells.

Rats and guinea pigs were exposed to 70 or 8 mg/m^3 of morpholine for 4 hours/day, 5 day/week for 4 months (Migukina, 1973). The investigators examined peripheral blood, lungs, liver, and kidneys, measured nervous system activity (by an undefined summary-threshold index), arterial pressure, and respiratory rate, and looked for chromosomal aberrations in bone marrow cells in the anaphase and telophase. The results of the study are summarized in Table 5-4. Morpholine had its most damaging effect on the spleen. Exposure to 70 mg/m^3 of morpholine resulted in destruction of the lymphoid structure. This effect was not reversible 1 month after exposure to morpholine ended. Based on indications of some mutagenic effect after exposure of 8 mg/m^3, the study used a somewhat arbitrary safety factor to recommend a maximum permissible concentration of 0.5 mg/m^3.

Morpholine is a highly irritating compound. Small amounts can burn the skin and eyes. Shea (1939) reported that application of undiluted morpholine to the fingertips produced an intense stinging sensation and cracking in the areas around the fingernail; even a 2% solution was not tolerated.

TABLE 5-4
Effects of Exposure to Morpholine on Rat and Guinea Pig

Concentration	Index of Effect	Species	Effect
70 mg/m^3	Nervous system activity	Rat	Initial increase, followed by return to control levels
		Guinea pig	Initial decrease; increase by end of 4 months
	Arterial pressure	Rat	Initial increase; decrease by 2nd month
	Peripheral blood	Rat	Increase in hemoglobin and red blood cell count; decrease in in leukocytes at 1st and 4th months
		Guinea pig	Decrease in hemoglobin and leukocyte
	Electrocardiogram	Rat	No change
	Organ function	Rat	No changes in liver, kidneys, and testes
		Guinea pig	No changes in kidneys and testes; change in liver function
	Morphology	Rat, guinea pig	Swelling of alveoli and atrophy of respiratory lymphatics; atrophy of lymphoid elements of the spleen even in animals killed 1 month after exposure ended
	Mutagenesis	Rat	Increase in number of chromosomal aberrations resulting from fragmentation
8 mg/m^3	Nervous system activity	Rat	Increase through 1st month of exposure
	Arterial pressure	Rat	Decrease by 2nd month
	Peripheral blood	Rat	Decrease in lymphocytes at 2nd month
		Rat, guinea pig	No changes in liver function
	Organ function	Rat	No changes in liver functionn
	Morphology	Rat, guinea pig	Decrease in size of lymph nodes of spleen; thus effect not observed in animals killed 1 month after exposure ended
	Mutagenesis	Rat	Increased in number of chromosomal aberrations although not significantly greater than spontaneous rate

---

[a] From Migukina, 1973, with permission.

Experiments (Shea, 1939) with rabbits and guinea pigs demonstrated the local as well as systemic effects from skin application of morpholine. Undiluted, unneutralized morpholine applied to the shaven skin of rabbits and guinea pigs caused deaths after 1 to 13 daily applications. Morpholine produced skin burns, necrosis, inflammation, and edematous derma. Systemic effects included congestion of the liver and spleen, fatty degeneration and necrosis of the liver, and necrosis of the kidney tubules. A diluted aqueous solution of morpholine produced similar systemic effects and deaths. In contrast, after 30 daily applications, an undiluted, neutralized morpholine solution has caused only a thickening of the derma.

Chronic Toxicity

Carcinogenicity

Shank and Newberne (1976) investigated the carcinogenic properties of morpholine through long-term feeding studies in rats and hamsters. Pregnant rats and hamsters were fed sodium nitrite, morpholine, and N-nitrosomorpholine (NMOR) in the diet from the time of conception until delivery. Offspring of both sexes were then randomly selected for the long-term studies, which also included studies of second-generation rats. The experiments were ended at 125 weeks for rats and 110 weeks for hamsters.

The dietary levels and incidence of cancer for the various experimental groups are summarized in Table 5-5. In groups 3, 7,

TABLE 5-5

Dietary Levels of Compounds Given to Rats and Hamsters and Incidence of Cancer[a]

	Dietary levels (ppm)			Incidence (%)				
				Rat			Hamster	
Test Group[b]	Sodium Nitrite	Morpholine	N-Nitroso-morpholine	Liver cell cancer	Liver angio-sarcoma	Lung angio-sarcoma	Liver cell cancer	Liver angio-sarcoma
1	0	0	0	0	0	0	4	0
2	1,000	0	0	1	0	0	0	3
3	0	1,000	0	3	0	2	0	0
4	1,000	1,000	0	97	14	23	31	0
5	1,000	50	0	59	5	6	0	0
6	1,000	5	0	28	12	8	0	0
7	50	1,000	0	3	2	1	0	5
8	5	1,000	0	1	2	1	0	0
9	50	50	0	2	1	1	0	3
10	5	5	0	1	2	2	0	0
11	0	0	5	58	15	9	0	0
12	0	0	50	93	21	20	6	6

---

[a] From Shank and Newberne, 1976, with permission

[b] Number of animals per group ranges from 94-172; F_1 and F_2 generations combined.

and 8, morpholine was the predominant additive in the standard diet. Rats had a small but higher incidence of liver cell cancer and angiosarcomas than did controls. These data, especially for group 3, might suggest that morpholine by itself may be a weak carcinogen. The investigators speculated, however, that tumor production in this group may have resulted from an unknown source of nitrate combining with morpholine to form the potent NMOR carcinogen, as was suspected to be the case in the other groups.

A similar study was performed by Greenblatt _et_ _al_. (1971), who investigated the chronic toxicity of NMOR in addition to the potential _in_ _vivo_ nitrosation of morpholine by sodium nitrite. Male and female Swiss mice (20 per group) were given 6.33 g/kg of morpholine concurrently with 1.0 g/kg of sodium nitrite indrinking water. Control animals received either morpholine alone, sodium nitrite alone, or were not treated. After 40 weeks, all survivors were killed. Animals treated with both morpholine and sodium nitrite showed a 57% (20 of 35) incidence of lung adenomas. Animals treated with either morpholine alone or sodium nitrite alone did not show different results than the untreated controls. Further studies are clearly needed to clarify the carcinogenic potential of morpholine.

The same study demonstrated the ability of dietary sodium ascorbate (vitamin C), given concurrently with morpholine and sodium nitrite, either to diminish the incidence of tumor formation or to increase the induction period. When 5.75, 11.5, or 23.0 g/kg of dietary sodium ascorbate was administered to A strain mice that also received 6.33 g/kg of morpholine and 2.0 g/kg of sodium nitrite in their drinking water, there was a 72-89% inhibition of adenoma formation (Greenblatt et al., 1971).

These observations were confirmed by Mirvish et al. (1976) with male Wistar rats. Groups of 40 rats were treated for 2 years with 10 g/kg of dietary morpholine and 3 g/liter of sodium nitrite in drinking water. In additon, one group of rats also received 22.7 g/kg of sodium ascorbate in their diet. The presence of ascorbate resulted in a longer tumor induction period (93 versus54 weeks) and a slightly lower tumor incidence (49% versus 65%). No pulmonary metastases were reported in the animals that received morpholine plus sodium nitrite.

A major concern is the ease by which morpholine can be nitrosated to form N-nitrosomorpholine (NMOR), which has been shown to be carcinogenic in rats, mice, and hamsters. After oral administration, NMOR produces both benign and malignant tumors of the liver and lungs in mice, of the liver, kidneys, and blood vessels in rats, and of the liver of hamsters. This subject has been extensively reviewed by the International Agency for Research on Cancer (1978). To date there is no evidence that NMOR is carcinogenic in humans.

A recent study by Iqbal *et al.* (1980) has shown a relationship between exposure to morpholine and nitrogen dioxide and the *in vivo* formation of NMOR. Mice (three or four per group) were gavaged with 2 mg of morpholine. The animals were then exposed to nitrogen dioxide at concentrations of 0.2 to 50 ppm for up to 4 hours in inhalation chambers. At various intervals during exposure, the mice were frozen and pulverized in liquid nitrogen. The powder was then analyzed for the presence of NMOR. There was a time-dependent NMOR yield relative to nitrogen-dioxide exposure--ranging from 370 ± 12.5 ng/mouse after 0.5 hours to 2,230 ± 138.6 ng/mouse after 4 hours. There was also a dose-dependent NMOR biosynthesis as a function of nitrogen dioxide exposure level (0.2-50 ppm). A 4-hour exposure of 0.2 ppm nitrogen dioxide resulted in 56 ± 6 ng NMOR per mouse, a 4-hour exposure of 50 ppm of nitrogen dioxide resulted in NMOR biosynthesis of more than 1,000 ng/mouse. Control levels of NMOR in mice gavaged with morpholine or distilled water and then exposed to air were less than 5.0 ng of NMOR per mouse. This study demonstrates the *in vivo* nitrosating potential of nitrogen oxides interacting with amines such as morpholine.

The results of this study were extended by Van Stee *et al.* (1980), who demonstrated excess tumor formation in male CD-1 mice. They exposed 35 animals per group to nitrogen dioxide (1-2 ppm) by inhalation while receiving 0.1% v/v morpholine in drinking water. After exposure for 30 weeks, the animals (and all the appropriate controls) received deionized water and room air until moribund or dead. The group that received the morpholine and nitrogen dioxide had an increased incidence of lung adenoma relative to controls (P = 0.056, 21.2%). Various other statistical tests all indicated a significant difference between the experimental and control groups, even with the relatively small sample sizes tested.

The results of these studies provide evidence that, exposure of mice to nitrogen dioxide and a secondary amine (morpholine) can lead to *in vivo* nitrosamine formation as well as to an increased incidence of tumor formation. Although the extrapolation to humans of the results of such studies is fraught with difficulties, these relatively low exposures give the results more relevance. Further work should be done to confirm these observations in other species, with larger sample sizes, and with simultaneous inhalation exposure to both morpholine and nitrogen dioxide. In addition, with the increased detection sensitivity of modern analytic methods, it should be possible to monitor humans exposed to similar conditions. Evidence of *in vivo* nitrosamine formation in humans exposed to morpholine and nitrogen dioxide would provide strong confirmation of the animal model and make major aspects of the extrapolation unnecessary.

Although NMOR is not known to be manufactured commercially, there is a potential for human exposure through the *in vivo* nitrosation of morpholine, although this route has not yet been demonstrated. However, Rounbehler *et al*. (1980) have shown the presence of several volatile nitrosamines, including NMOR, in the interiors of 1979-model automobiles. NMOR was detected at levels between a trace and 2.5 $\mu g/m^3$ (mean of 0.65 $\mu g/m^3$) in the interiors of 16 of 38 automobiles tested. This nitrosamine, as well as several others detected, probably account for the "new car smell."

Mutagenicity of Morpholine

Morpholine has been tested in *Salmonella* mutagenicity systems, primarily the host-mediated assay, and in a transplacental mutagenicity assay. It is nonmutagenic in the absence of a nitrosating agent.

Bacteria. Given to mice in the host-mediated assay with *Salmonella*, morpholine was not mutagenic. Zeiger and Legator (1971) administered up to 500 mg/kg of morpholine by gavage or intramuscularly to male mice and gave intraperitoneal injection of *Salmonella typhimurium* G-46 as the indicator organism. No mutagenic activity was observed.

Braun *et al*. (1977) treated male mice with morpholine at 1.45 to 2.90 mmol/kg by gavage in the intraperitoneal host-mediated assay. No mutagenic activity was seen in the indicator organism, *S. typhimurium* TA1950. In the same study, administration of equimolar levels of sodium nitrite (2.175 and 2.90 mmol/kg) produced a mutagenic response. This response was observed only when the sodium nitrite was administered along with the morpholine or 10 minutes later. Administration of sodium nitrite 10 minutes before administration of morpholine produced no response. At levels up to 2.9 mmol/kg, sodium nitrite by itself did not produce mutagenic activity. Using the intrahepatic host-mediated assay, Edwards *et al.* (1979) demonstrated the nonmutagenicity of morpholine in *S. typhimurium* TA1530 recovered from the livers of female mice receiving 4 mg/kg of morpholine by gavage. When 4 mg/kg of morpholine was administered immediately before administration of 120 mg/kg of sodium nitrite, a mutagenic response was observed. The authors estimated the relative conversion of morpholine to NMOR at 12.3-19.8%, using morpholine levels doses ranging from 4 to 40 mg/kg and sodium nitrite levels of 120 mg/kg and comparing the mutagenic responses to that obtained from pure NMOR. Sodium thiocyanate (120 mg/kg), a catalyst of nitrosation, enhanced NMOR formation as measured by mutagenicity; doses of 4 or 20 mg/kg did not affect the response. Ascorbic acid, an inhibitor of nitrosation, inhibited the mutagenic response of 40 mg of morpholine and 120 mg of sodium nitrite per kilogram at levels of 120 and 360 mg/kg, but not at the 40-mg/kg level.

Practical-grade morpholine was tested *in vitro* for mutagenicity in *Salmonella* at EG&G Mason Research Institute in Rockville, Md. through the Environmental Mutagenesis Test Development Program of the National Institute for Environmental Health Sciences (NIEHS). The investigators used a preincubation modification of the *Salmonella*/microsome test using Aroclor-induced rat and Syrian hamster liver S-9 and *Salmonella* strains TA98, TA100, TA1535 and TA1537. No mutagenicity was observed either with or without S-9 at doses ranging up to 10.0 mg/plate (S. Haworth, personal communication, 1980).

Mammals. Pregnant golden hamsters were administered morpholine by gavage, with and without sodium nitrite (Inui *et al*., 1979). Morpholine alone, at doses of 500 mg/kg, did not induce chromosomal aberrations, micronuclei, 8-azaguanine- or ouabain-resistant mutants, or transformation in cells from exposed embryos. The results from the combination of morpholine and sodium nitrite are discussed below.

Mutagenicity of N-Nitrosomorpholine

The results of genetic studies with NMOR are summarized in Table 5-6. The substance was found to induce gene mutations in all bacterial and mammalian cell culture systems by using a source of exogenous metabolism. NMOR is presumably the mutagen detected in the host-mediated assay in animals given both morpholine and sodium nitrite. Chromosomal aberrations were also observed in cultured mammalian cells. NMOR is active in whole animal systems, producing gene and chromosomal mutations in *Drosophila*. It is also responsible for DNA damage, as measured by the production of single- and double-strand DNA breaks in liver and the alkylation of nucleotides in DNA. There are, however, no adequate studies on the induction of chromosomal mutations or heritable effects in mammals.

Bacteria (In Vitro). Only in the presence of liver homogenate is NMOR mutagenic *in vitro* for *Salmonella* and *Escherichia coli*. Base-pair substitution mutations are induced with no evidence of induction of frame-shift mutations. NMOR was active in *Salmonella* TA1535 and TA1530 in both plate and suspension tests, using

Table 5-6
Results of Genetic Studies with N-nitrosomorpholine

Test System	Species/Strain or Cells Tested	Result	Reference
Gene mutation	*S. typhimurium* TA1535	+	Zeiger and Sheldon, 1978
	S. typhimurium TA1535	+	Gomez *et al.*, 1974
	S. typhimurium TA1530	+	Bartsch *et al.*, 1976
	S. typhimurium TA1950	+	Fonshtein *et al.*, 1976
	S. typhimurium G-46 (host-mediated assay)	+	Zeiger and Legator, 1971
	S. typhimurium TA1530 (host-mediated assay)	+	Zeiger, 1973, 1975
	S. typhimurium TA1950 (host-mediated assay)[a]	+	Fonshtein *et al.*, 1976
	S. typhimurium TA1530 (host-mediated assay)[a]	+	Braun *et al.*, 1977
	S. typhimurium TA1530 (host-mediated assay)	+[b]	Edwards *et al.*, 1979
	S. typhimurium TA1535	+[c]	Charnley and Archer, 1977
	E. coli B/R	–	Henke *et al.*, 1964
	E. coli SD-B(TC)	+	Nakajima *et al.*, 1974
	E. coli WU3610	+	Elespuru and Lijinsky, 1976
	E. coli WU3610	+[b]	Elespuru, 1978
	E. coli C600, A58	–[c]	Neale, 1972
	BHK-21 cells ($8AG^R$)	+	Kimble *et al.*, 1973
	V79 cells ($8AG^R$)	+	Kuroki *et al.*, 1977
	V79 cells (IAG^R, ouq^R)	+	Drevon *et al.*, 1977
	V79 cells ($6TG^R$)	+[d]	Jones and Huberman, 1980
	Drosophila (sex-linked recessive lethal)	+	Henke *et al.*, 1964
	Drosophila (sex-linked recessive lethal)	+	Vogel, 1977
	Golden hamster embryo ($8AG^R$, oua^R)	+[e]	Inui *et al.*, 1979
Chromosomal mutation	BHK-21 cells	+	Kimble *et al.*, 1973, 1975
	Rat bone marrow	+/–[f]	Sauro *et al.*, 1973
	Rat lymphocytes *in vivo*	+[g]	Newton *et al.*, 1977
	Mouse dominant lethal	–[h]	Parkin *et al.*, 1973
	Drosophila (II/III translocations)	+	Henke *et al.*, 1964
	Golden Hamster embryo (aberrations)	+[e]	Inui *et al.*, 1979
	Golden Hamster embryo (micronuclei)	+[e]	Inui *et al.*, 1979

Plants	*Arabidopsis thaliana*	-	Veleminsky and Gichner, 1968
DNA damage/repair	Rat, *in vivo* (7-MeG excretion)	-	Weyland *et al.*, 1972
	Rat, *in vivo* (DNA strand breaks in liver)	+	Stewart and Farber, 1973
	Rat, *in vivo* (DNA alkylation)	+	Stewart *et al.*, 1974
	Chang liver cells (BUdR incorporation)	+[c]	Kimble *et al.*, 1974
	BHK-21/C 13 cells (UDS)	+[c]	Kimble *et al.*, 1975
	Rat, *in vivo* (DNA alkylation)	+	Kleihues and Margison, 1976
	Mouse, *in vivo* (DNA strand breaks in liver)	+	Schwarz *et al.*, 1979
	Rat primary hepatocytes (TdR incorp.)	+	Williams and Laspia, 1979
Other nuclear damage	Rat, *in vivo* (liver mitotic abnormalities)	+	Romen and Bannasch, 1972

---

[a] Following administration of NMOR plus sodium nitrite.
[b] Deuterated NMOR.
[c] Without an exogenous metabolic activation system.
[d] Using a feeder cell layer for metabolism.
[e] Transplacental.
[f] Inconsistent results.
[g] Weak, questionable positive.
[h] Decreased fertility.

uninduced or phenobarbital-induced rat and mouse liver (Bartsch *et al.*, 1976; Gomez *et al.*, 1974; Zeiger and Sheldon, 1978) and in *E. coli* in suspension using Aroclor-induced or uninduced rat S-9 (Elespuru, 1978; Elespuru and Lijinsky, 1976; Nakajima *et al.*, 1974). No mutagenicity was seen for *E. coli* (Henke *et al.*, 1964) or induction of λ-phage from *E. coli* in the absence of exogenous metabolic activation.

In a comparison of uninduced rat and mouse S-9 in suspension and on the standard plate test using TA1535, mouse S-9 produced a consistently higher mutagenic response than did rat liver (Zeiger and Sheldon, 1978). Phenobarbital-induced rat liver S-9 produced a higher response in the plate test than did human liver S-9; rat and human lung S-9 produced no mutagenic activity (Bartsch *et al.*, 1976). NMOR mutagenicity for *E. coli* WU3610 and *S. typhimurium* TA1535 was depressed when the alpha position of NMOR was deuterated, leading to the conclusion that an enzymatic attack on the alpha position is necessary for the activation of NMOR (Charnley and Archer, 1977; Elespuru, 1978).

Bacteria (Host-Mediated). NMOR is mutagenic for *Salmonella* G46 and TA 1530 in the intraperitoneal host-mediated assay in the mouse (Fonshtein *et al.*, 1976; Zeiger, 1975; Zeiger and Legator, 1971) when administered by intramuscular injection or by gavage. Alterations in both the diet and amino acetonitrile pretreatment affected the mutagenic response. Maintenance of the mice on a complete semisynthetic diet depressed the mutagenicity of NMOR as compared with results from the corresponding chow diet control. Mice maintained on the semisynthetic diet and then put on a protein-free diet for 8 days had a further depressed mutagenic response. Starvation or an all-casein diet for 24 hours produced an enhanced mutagenic response (Zeiger, 1975). Aminoacetonitrile, an inhibitor of liver microsomal metabolism, administered in a dosage of 20 to 200 mg/kg, apparently produced a dose-related depression of NMOR mutagenicity (Zeiger, 1973).

The formation of NMOR from morpholine and sodium nitrite has been successfully followed, using both the intraperitoneal and intrasanguineous host-mediated assays with S. typhimurium TA1950 and TA1530. When mice were treated with equimolar concentrations of morpholine and sodium nitrite by gavage, mutagenicity was observed with morpholine administered with or before the administration of sodium nitrite. Morpholine administered 10 minutes after sodium nitrite produced no mutagenicity (Braun et al., 1977). In the intrasanguineous host-mediated assay, mutagenicity was seen in TA1530 recovered from the livers of mice treated by gavage with morpholine and sodium nitrite. Ascorbic acid, which inhibits nitrosation, decreased the mutagenic response; thiocyanate, which enhances the nitrosation reaction, enhanced mutagenicity (Edwards et al., 1979).

Mammalian Cells in Culture. NMOR induces gene mutations in cultured mammalian cells. Equivalent cytotoxicities were observed (Kuroki et al., 1977) in V79 cells treated with NMOR with or without an S-9 fraction from phenobarbital-treated rats. However, 8-azaguanine resistance was produced only in the presence of S-9. In another study (Jones and Huberman, 1980), hepatocytes from phenobarbitone- or benz(a)anthracene-induced rat liver were used for metabolic activation by cocultivation with V79 cells. Both ouabain- and 6-thiouguanine-resistant mutants were induced at nontoxic levels In BHK 21 cells, NMOR induced 8-azaguanine resistance and chromosomal aberrations, predominantly dicentrics (Kimble _et al._, 1973). An exogenous source of metabolic activation was not used in this study.

Mammalian Cells In Vivo. Single oral doses of NMOR given to rats produced inconsistent levels of chromatid breaks and gaps in aspirates from bone marrow. Chronic dietary administration of morpholine and sodium nitrite produced no detectable cytogenetic effects in bone marrow (Sauro _et al._, 1973). Administration of NMOR in drinking water resulted in mitotic abnormalities and an increased mitotic index in rat liver (Romen and Bannasch, 1972).

After rats were given 200 to 300 mg/kg of NMOR, their lymphocytes were removed, cultured, and examined for chromosomal aberrations (Newton _et al._, 1977). Increases in abnormal metaphases were observed at 250 mg/kg in rats killed at 20 hours and at 300 mg/kg in rats killed at 3 or 20 hours. These aberrations were primarily gaps and breaks. The increases in single chromatid or isolocus breaks were not consistent with dosage or with each other within the same dosage. Two chromatid exchanges were seen in all treatment groups.

In a mouse dominant lethal test, administration of 50 and 100 mg/kg of NMOR reduced mating incidence 2 and 3 weeks after treatment. No dominant lethal effects were observed 3 to 8 weeks after treatment. A dosage of 35 mg/kg resulted in normal mating and no dominant lethal effects during the first 3 weeks after treatment (Parkin *et al.*, 1973). Intraperitoneal treatment of rats with 400 mg/kg of NMOR produced a disruption of liver nuclear structure and a breakdown of nucleolar structure (Stewart *et al.*, 1975).

In a recent study by Inui *et al.* (1979), 500 mg of morpholine and 500 mg/kg of sodium nitrite were administered to golden hamsters by gavage on day 11 or 12 of pregnancy. The levels of NMOR recovered from the stomachs of these animals ranged from a high of 1.94 mg after 1 hour to 0.59 mg 24 hours after treatment. Approximately 5.6 μg of *N*-nitrosodiethylamine (NDEA) was recovered from animals treated only with sodium nitrite. Twenty-four hours after treatment, the embryos were excised and the cells grown and analyzed for chromosomal aberrations, micronuclei, 8-azaguanine and ouabain resistance, and transformation.

Cells from animals treated with the combination of morpholine and sodium nitrite had increased numbers of chromatid gaps and breaks (which the authors concluded were significant) and increased numbers of micronuclei. There were also increased

8-azaquanine- and ouabain-resistant cell frequencies and an increased rate of morphologic transformation. Cells from the transformed colonies produced tumors when inoculated into cheek pouches of young golden hamsters.

In all end points, sodium nitrite alone induced increases that were lower than, but in the same range as, the responses induced by the substance in combination with morpholine. The administration of sodium nitrite was considered to produce positive results for all end points. Morpholine alone produced negative results for all end points. NMOR produced positive results for all end points. However, sodium nitrite in doses of 100 to 200 mg/kg produced a higher response than did NMOR of 100 mg/kg for all end points except chromosomal aberrations, and a higher response than did 200 mg/kg of NMOR in the micronucleus and cell transformation tests. The morpholine-plus-NMOR response was higher than that produced by NMOR alone at 200 mg/kg for micronucleus and cell transformation tests, and was equivalent for ouabain resistance.

These comparisons are difficult to interpret. The total dose of NMOR given to those animals receiving the combination of morpholine plus sodium nitrite cannot be calculated, but it is difficult to conceive that it approached 100-200 mg/kg. Yet, the response obtained suggests that if this level of NMOR was formed, further study is necessary. Also, the strong responses seen with sodium nitrite alone call for further explanation and more study because the results imply that sodium nitrite is a more potent mutagen *in vivo* than is NMOR. All other data indicate that sodium nitrite has little, if any, genetic activity toward anything other than microorganisms.

DNA Damage. Rats receiving 400 mg/kg carbon-14-labeled NMOR intraperitoneally (IP) were found to have labeled 7-(2-hydroxyethyl)-guanine plus five other labeled adducts in liver DNA (Stewart *et al.*, 1974). Urine of rats administered a single dose of NMOR (120 mg/kg, IP) did not contain 7-methylguanine when sampled for up to 21 days (Weyland *et al.*, 1972).

Partially hepatectomized rats were treated with carbon-14-thymidine to label their liver DNA, then injected intraperitoneally with NMOR at 100 mg/kg. Four hours after treatment, there was a shift in the sedimentation of DNA on alkaline gradients consistent with the introduction of single-strand DNA breaks. Reduction of the dosage to 10 mg/kg resulted in only a slight change in sedimentation rate as compared to results produced by the larger dose. The DNA breaks were repaired gradually over a period of 14 days. Double-strand breaks were also induced, as measured by neutral sucrose gradients. This type of break was not seen following administration of NDEA. Morpholine, itself, produced no measurable DNA damage (Stewart and Farber, 1973).

Intravenous administration of 50 mg/kg of NMOR produced a small increase over background of 0^6-methylguanine in rat liver DNA, or a lesion that was recognized by the 0^6-methylguanine excision system (Kleihues and Margison, 1976). Mouse liver DNA strand breakage was also observed via akaline elution technique 4 and 12 hours after treatment with 100 mg/kg of NMOR by intraperitoneal injection (Schwarz *et al*., 1979).

In vitro studies with Chang liver cells and BHK-21 cells in culture resulted in an increase in DNA repair synthesis after treatment with NMOR at 100 μg/ml or less (Kimble *et al*., 1974, 1975). Using primary rat hepatocytes in culture, Williams and Laspia (1976) showed an increase in DNA repair synthesis at 10^{-2} and 10^{-3} mol. NMOR.

Drosophila. NMOR is capable of inducing sex-linked recessive lethal mutations in *Drosophila melanogaster* sperm and spermatids after feeding (Vogel, 1977). Sex-linked recessive lethals and II/III translocations occur after injection of NMOR (Henke, *et al*. 1964).

Plants. Treatment of *Arabidopsis thaliana* seeds with NMOR for 24 hours did not induce either sterility or mutations, expressed as the presence of M_1 siliquae (Veleminsky and Gichner, 1968). Using these treatment conditions, a number of aliphatic nitrosamines in the same study were strongly mutagenic.

Teratogenicity

There were no data from which to evaluate the potential teratogenicity or reproductive toxicity of morpholine.

CONCLUSIONS

The carcinogenic potential for morpholine alone is somewhat equivocal because it is not possible to know the extent to which morpholine is nitrosated *in vivo*. What does appear more certain is that the carcinogenicity of morpholine is greatly enhanced when it is administered concurrently with a source of nitrite (e.g. sodium nitrite or nitrogen dioxide), which allows nitrosation to NMOR to occur. That nitrosation *in vivo* occurs is demonstrated by the inhibition of the carcinogenic effects by the presence of an antioxidant (such as sodium ascorbate) in the diet.

The potential for this *in vivo* nitrosation of morpholine (or of any other amine) needs further and immediate attention. In addition to research on experimental animals, human exposure should be carefully monitored. Individuals occupationally exposed to morpholine should be studied to determine if concurrent exposure to nitrogen dioxide and other nitrosating agents results in an increased cancer incidence or perhaps in an elevated level of serum NMOR. In addition, more data on noncarcinogenic toxicity, teratogenicity, and reproductive effects should be obtained.

Because NMOR can be formed *in vivo* from ingested morpholine and nitrite, morpholine for this reason should be considered as hazardous as NMOR. The roles of tobacco smoke and diet as secondary nitrite sources should also be investigated. This may also provide a unique opportunity to study and (perhaps to quantify) the effects of synergism in humans. Properly designed and imaginative epidemiologic studies of this kind may shed light on the current confused situation concerning occupational exposure to chemicals and cancer incidence.

Although morpholine has produced negative results in the few studies in which it was examined by itself, the primary concern remaining is its ability to be nitrosated readily to form the mutagen/carcinogen NMOR. *In vivo* studies have demonstrated the formation of a mutagen, presumably NMOR, following the administration of morpholine plus sodium nitrite.

NMOR is well-established as a mutagen. It induces point mutations in cells *in vitro* and in *Drosophila* as well as chromosomal aberrations in cultured cells. This latter effect is observed even in the absence of an S-9 preparation. In rodents, NMOR induces DNA damage in liver and low levels of chromosomal aberrations in bone marrow. It does not appear to reach the testes in an active form, although the results of the single negative rat dominant lethal test are not sufficient to conclude that NMOR cannot induce heritable effects in rats.

REFERENCES

Production, Uses, Exposure

Anonymous. 1980. Air products may become morpholine producer. Chem. Eng. News 58(8):28.

Chemical Marketing Reporter. 1974. Chemical profiles: Morpholine. Chem. Mark. Rep. 205(22):9, June 3. Schnell Publishing Co., New York.

Code of Federal Regulations. 1980. Title 21, Part 172. Food additives permitted for direct addition to food for human consumption. Office of the Federal Registrar, National Archives and Records Service, General Services Administration, Washington, D.C.

Food and Drug Administration. 1980. Information received under 21 CFR Part 720 by the Division of Cosmetic Technology, Food and Drug Administration, Washington, D.C.

Kirk-Othmer Encyclopedia of Chemical Technology. 1978. Third Edition, Vol. 2. Martin Grayson, exec. ed. and David Eckroth, assoc. ed. A Wiley-Interscience Publication. John Wiley & Sons, New York. 1,036 pp.

McCain, J.C., and J. M. Peck. 1976. The Toxicity of Selected Chemicals Used in Power Generating Stations to Hawaiian Fishes. University of Hawaii, Honolulu.

Occupational Safety and Health Administration. 1980. Toxic and Hazardous Substances. 29 CFR 1910.1000. Office of the Federal Register, Washington, D.C.

Science Applications, Inc. 1980. Human Exposure to Atmospheric Concentrations of Selected Chemicals. Draft Report No. EF-156R to U. S. Environmental Protection Agency, March, 1980.

Singer, G.M., and W. Lijinsky. 1976. Naturally occurring nitrosable compounds. I. Secondary amines in foodstuffs. J. Agri. Food Chem. 24:550-553.

Stanford Research Institute International . 1975. Directory of Chemical Producers-U.S.A. Supplement, Stanford Research Institute International, Menlo Park, Calif.

U.S. International Trade Commission. 1978. Synthetic Organic Chemicals: United States Production and Sales, 1977. USITC Publication 920. U.S. International Trade Commission, Washington, D.C. 417 pp.

Analytic Methods

Burenko, T.S., E.G. Zhuravlev, and T.A. Miklashevich. 1977. Determination of morpholine in the air. Gig. Tr. Prof. Zabol. No. 3:55-56. [Chem. Abs. 86:194286s, 1977]

Fajen, J.M., G.A. Carson, D.P. Rounbehler, T.Y. Fan, R. Vita, U.E. Goff, M.H. Wollf, G.S. Edwards, D.H. Fine, V. Reinhold, and K. Biemann. 1979. N-Nitrosamines in the rubber and tire industry. Science 205:1262-1264.

Karweik, D.H., and C.H. Meyers. 1979. Spectrophotometric determination of secondary amines. Anal. Chem. 51:319-320.

Singer, G.M., and W. Lijinsky. 1976a. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. J. Agric. Food Chem. 24:550-553.

Singer, G.M., and W. Lijinsky. 1976b. Naturally occurring nitrosatable amines. II. Secondary amines in tobacco and cigarette smoke condensate. J. Agric. Food Chem. 24:553-555.

Tombropoulos, E.G. 1979. Micromethod for the gas chromatographic determination of morpholine in biological tissues and fluids. J. Chromatogr. 164:95-99.

Health Effects

American Conference of Governmental Industrial Hygienists. 1974. Pp. 175-176 in Documentation of the Threshold Limit Values for Substances in Workroom Air with Supplements for those Substances Added or Changed since 1971. American Conference of Governmental Industrial Hygienists, Third Edition, Second Printing. Cincinnati.

Bartsch, H., A. Camus, and C. Malaveille. 1976. Comparative mutagenicity of N-nitrosamines in a semisolid and in a liquid incubation system in the presence of rat or human tissue fractions. Mutat. Res. 37:149-162.

Braun, R., J. Schoneich, and D. Ziebarth. 1977. In vivo formation of N-nitroso compounds and detection of their mutagenic activity in the host-mediated assay. Cancer Res. 37:4572-4579.

Charnley, G., and M.C. Archer. 1977. Deuterium isotope effect in the activation of nitrosomorpholine into a bacterial mutagen. Mutat. Res. 46:265-268.

Drevon, C., T. Kuroki, and R. Montesano. 1977. Microsome-mediated mutagenesis of a Chinese hamster cell line by various chemicals. Pp. 207-213 in D. Scott, B.A. Bridges, and F.H. Sobels, eds. Progress in Genetic Toxicology. Elsevier/North-Holland, New York.

Edwards, G., W.Z. Whong, and N. Speciner. 1979. Intrahepatic mutagenesis assay: A sensitive method for detecting N-nitromorpholine and in vivo nitrosation of morpholine. Mutat. Res. 64:415-423.

Elespuru, R.K. 1978. Deuterium isotope effects in mutagenesis by nitroso compounds. Mutat. Res. 54:265-270.

Elespuru, R.K., and W. Lijinsky. 1976. Mutagenicity of cyclic nitrosamines in Escherichia coli following activation with rat liver microsomes. Cancer Res. 36:4099-4101.

Fonshtein, L.M., S.K. Abilev, A.M. Zekhnev, and A.A. Shapiro. 1976. A study of the effects of the mutagenic metabolic activation of N-nitrosomorpholine on microorganisms. Sov. Genet. 12:610-615. [Translation of Genetika 12(5):119-125 1976]

Gomez, R.F., M. Johnston, and A.J. Sinsky. 1974. Activation of nitrosomorpholine and nitrosopyrrolidine to bacterial mutagens. Mutat. Res. 24:5-7.

Greenblatt, M., S. Mirvish, and B.T. So. 1971. Nitrosamine studies: Induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. J. Natl. Cancer Inst. 46:1029-1034.

Grodetskaya, N.S., and N.M. Karamzina. 1973. Initial reactions by the organism to the effects of industrial substances in minimal effective concentrations (Lim_{ac}, Lim_{ch}). Pp. 9-18 in A.A. Letavet and I V. Sanotskiy, eds. The Toxicology of New Industrial Chemical Substances, Issue No. 13. USSR Academy of Medical Sciences, Meditsina, Moscow. [Translated from the Russian]

Henke, R., G. Hohne, and H.A. Kunkel. 1964. Uber die mutagene Wirkung von Rontgenstrahlen, N-nitroso-N-methyl-urethan and N-nitroso-morpholin bei Drosophila melanogaster. Biophysik 1:418-421.

Industrial Bio-Test Laboratories, Inc. 1970. Bio-Fax Data Sheet: Morpholine. Industrial Bio-Test Laboratories, Inc., Northbrook, Ill.

International Agency for Research on Cancer. 1978. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 17. Some N-Nitroso Compounds. International Agency for Research on Cancer, Lyon.

International Labour Office. 1972. Pp. 915-916 in Encyclopaedia of Occupational Health and Safety, Volume II. International Labour Office, Geneva.

Inui, N., Y. Nishi, M. Taketomi, M. Mori, M. Yamamoto, T. Yamada, and A. Tanimura. 1979. Transplacental mutagenesis of products formed in the stomach of golden hamsters given sodium nitrite and morpholine. Int. J. Cancer 24:365-372.

Iqbal, Z.M., K. Dahl, and S.S. Epstein. 1980. Role of nitrogen dioxide in the biosynthesis of nitrosamines in mice. Science 207:1475-1477.

Ivanov, N.G., and A.L. Germanova. 1973. Comparative sensitivity of animal and man to the effects of irritant toxins. Pp. 36-41 in A.A. Letavet and I.V. Sanotskiy, eds. The Toxicology of New Industrial Chemical Substances, Issue No. 13. USSR Academy of Medical Sciences, Meditsina, Moscow. [Translated from the Russian]

Ivanov, N.G., A.L. Germanova, A.M. Klyackina, G.G. Maksimov, and V.S. Pozdnyakov. 1973. Comparative evaluation of the methods of finding the irritating action of industrial toxins and the calculations of their maximum permissible concentrations in the air of a work zone. Pp. 19-28 in A.A. Letavet and I.V. Sanotskiy, eds. The Toxicology of New Industrial Chemical Substances, Issue No. 13. USSR Academy of Medical Sciences, Meditsina, Moscow. [Translated from the Russian]

Jones, C.A., and E. Huberman. 1980. A sensitive hepatocyte-mediated assay for the metabolism of nitrosamines to mutagens for mammalian cells. Cancer Res. 40:406-411.

Kimble, C.E., A.J. Sinsky, and P. Gorczyca. 1973. Mutagenesis of BHK-21 cells by N-nitroso compounds and ultraviolet light. In Vitro 8:442-443, abstract no. 149.

Kimble, C.E., P.A. Gorczyca, and A.J. Sinskey. 1974. Repair of lesions induced in liver cell DNA by N-nitroso compounds as measured by the bromodeoxyuridine photolysis method. Mutat. Res. 24:35-39.

Kimble, C.E., P.A. Gorczyca, and R.G. Reynolds. 1975. N-Nitrosomorpholine and N-nitrobutylamine-stimulated DNA synthesis in BHK-21/C13 cells. Mutat. Res. 31:153-161.

Kleihues, P., and G.P. Margison. 1976. Exhaustion and recovery of repair excision of O^6-methylguanine from rat liver DNA. Nature 259:153-155.

Kuroki, T., C. Drevon, and R. Montesano. 1977. Microsome-mediated mutagenesis in V79 Chinese hamster cells by various nitrosamines. Cancer Res. 77:1044-1050.

Lam, H.F., and E.W. Van Stee. 1978. A re-evaluation of the toxicity of morpholine. Fed. Proc. 37:679, abstract no. 2459.

Migukina, N.V. 1973. Evaluation of the danger of morpholine during chronic action. Pp. 87-94 in A.A. Letavet and I.V. Sanotskiy, eds. The Toxicology of New Industrial Chemical Substances, Issue No. 13. USSR Academy of Medical Sciences, Meditsina, Moscow. [Translated from the Russian]

Mirvish, S.S., A.F. Pelfrene, H. Garcia, and P. Shubik. 1976. Effect of sodium ascorbate on tumor induction in rats treated with morpholine and sodium nitrite, and with nitrosomorpholine. Cancer Lett. 2:101-108.

Nakajima, T., A. Tanaka, and K.I. Tojyo. 1974. The effects of metabolic activation with rat liver preparations on the mutagenicity of several N-nitrosamines on a streptomycin-dependent strain of *Escherichia coli*. Mutat. Res. 26:361-366.

Neale, S. 1972. Effect of pH and temperature on the nitrosamide-induced mutation in *Escherichia coli*. Mutat. Res. 14:155-164.

Newton, M.F., B. Bahner, and L.J. Lilly. 1977. Chromosomal aberrations in rat lymphocytes treated *in vivo* with 1-phenyl-3,3-dimethyltriazene and N-nitrosomorpholine. A further report on a possible method for carcinogenicity screening. Mut. Res. 21: 155-161.

Parkin, H., H.B. Waynforth, and P.N. Magee. 1973. The activity of some nitroso compounds in the mouse dominant lethal assay. I. Activity of N-nitroso-N-methylurea, N-methyl-N-nitroso-N'-nitroquanidine and N-nitrosomorpholine. Mutat. Res. 21:155-161.

Pennsylvania Department of Health, Division of Occupational Health. 1967-1969. Supplement #1 to Short Term Limits for Exposure to Airborne Contaminants: A Documentation, 1967-1969. Pennsylvania Department of Health, Division of Occupational Health, Harrisburg, Pa.

Romen, W., and P. Bannasch. 1972. Karyokinesis and nuclear morphology during hepatocarcinogenesis. I. Mitoses and mitotic abnormalities in hepatocytes and hepatoma cells of the nitrosomorpholine-intoxicated rat liver. Virchows Arch. B. 11:24-33. [In German, English summary]

Rounbehler, D.P., J. Reisch, and D.H. Fine. 1980. Nitrosamines in new motor-cars. Food Cosmet. Toxicol. 18:147-151.

Sauro, F., L. Friedman, and S. Green. 1973. Biochemical, mutagenic and pathological effects of nitrosamines in rats. Toxicol. Appl. Pharmacol. 25:449, abstract no. 27.

Schwarz, M., J. Hummel, K.E. Appel, R. Richart, and W. Kunz. 1979. DNA damage induced in vivo evaluated with a non-radioactive alkaline elution technique. Cancer Lett. 6:221-226. [Cumul. Ind. Med. 20:4078, 1979]

Shank, R.C., and P.M. Newberne. 1976. Dose-response study of the carcinogenicity of dietary sodium nitrite and morpholine in rats and hamsters. Food Cosmet. Toxicol. 14:1-8.

Shea, T.E., Jr. 1939. The acute and sub-acute toxicity of morpholine. J. Ind. Hyg. Toxicol. 21:236-245.

Smyth, H.F., Jr., C.P. Carpenter, C.S. Weil, and U.C. Pozzani. 1954. Range-finding toxicity data, List V. AMA Arch. Ind. Hyg. Occup. Med. 10:61-68.

Stewart, B.W., and E. Farber. 1973. Strand breakage in rat liver DNA and its repair following administration of cyclic nitrosamines. Cancer Res. 33:3209-3215.

Stewart, B.W., P.F. Swann, J.W. Holsman, and P.N. Magee. 1974. Cellular injury and carcinogenesis. Evidence for the alkylation of rat liver nucleic acids in vivo by N-nitrosomorpholine. Z. Krebsforsch. 82:1-12.

Stewart, B.W., R.M. Hicks, and P.N. Magee. 1975. Acute biochemical and morphological effects of N-nitrosomorpholine in comparison to dimethyl- and diethylnitrosamine. Chem. Biol. Interact. 11:413-429.

Van Stee, E.W., G.A. Boorman, and J.C. Haseman. 1980. Pulmonary adenomas in mice exposed to NO_2 by inhalation and morpholine by ingestion. Pharmacologist 22(3):158 (abstract).

Veleminsky, J., and T. Gichner. 1968. The mutagenic activity of nitrosamines in Arabidopsis thaliana. Mutat. Res. 5:429-431.

Vogel, E. 1977. Identification of carcinogens by mutagen testing in Drosophila: The relative reliability for the kinds of genetic damage measured. Pp. 1483-1497 in H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds. Origins of Human Cancer. Book C. Human Risk Assessment. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Weyland, P., H.J. Gross, and H. Dannenberg. 1972. Urinary 7-methyl guanine excretion of the rat after a single application of different carcinogens. Z. Krebsforsch. Klin. Onkol. 77:141-149. [in German] [Chem. Abs. 77:57403c, 1972]

Williams, G.M., and M.F. Laspia. 1979. The detection of various nitrosamines in the hepatocyte primary culture/DNA repair test. Cancer Lett. 6:199-206. [Cumul. Ind. Med. 20:4695, 1979]

Zaeva, G.N., L.A. Timofievskaya, L.A. Bazarova, and N.V. Migukina. 1968. Comparative toxicity of a group of cyclic imino compounds. Toksikol. Nov. Prom. Khim. Vestchestv. No. 10:25-35. [Chem. Abstr. 71:47804w 1969]

Zeiger, E. 1973. Some factors affecting the host-mediated assay response. Environ. Health Perspect., Experimental Issue No. 6:101-109.

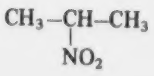
Zeiger, E. 1975. Dietary modifications affecting the mutagenicity of N-nitroso compounds in the host-mediated assay. Cancer Res. 35:1813-1818.

Zeiger, E., and M. Legator. 1971. Mutagenicity of N-nitrosomorpholine in the host-mediated assay. Mutat. Res. 12:469-471.

Zeiger, E., and A.T. Sheldon. 1978. The mutagenicity of heterocyclic N-nitrosamines for Salmonella typhimurium. Mutat. Res. 57:1-10.

Chapter 6

2-NITROPROPANE



$$CH_3-\underset{NO_2}{\underset{|}{C}H}-CH_3$$

2-Nitropropane (2-NP) is a colorless liquid with a moderately high vapor pressure (13 mm Hg at 20°C). It remains liquid over a wide range of temperatures. The melting point is -93°C; the boiling point, 120°C.

The compound is produced commercially through the reaction of propane and nitric acid at 370-450°C and 8-12 atm. The end products (including 1-nitropropane) are separated in fractionation towers.

PRODUCTION

The sole commercial producer of 2-NP worldwide is the International Minerals and Chemicals Corporation. Its main plant (in Sterlington, La.) has a capacity of 38,547 mt/year of basic nitroparaffins (Stanford Research Institute International, 1979). The four nitroparaffins (nitromethane, nitroethane, 1-nitropropane, and 2-NP) are produced in fixed weight ratios by the manufacturing process. Nitromethane comprises 28% of the products; nitroethane, 8%; 1-nitropropane, 18%; and 2-NP, 46% (Anonymous, 1976).

The National Institute for Occupational Safety and Health (1977) has estimated that 14,000 mt of 2-NP were produced in the United States in 1977. The International Minerals and Chemicals Corporation's annual production figure for basic nitroparaffins, as stated in its annual report for 1977, was 34,000 mt. If one assumes that 46% was 2-NP, then 16,000 mt would have been produced in 1977. The company did not publish production figures for basic nitroparaffins in its 1978 annual report, but the 1979 annual report gave a figure of 30,000 mt of basic nitroparaffins for 1979. If one assumes that 46% was again 2-NP, then 14,000 mt of 2-NP were produced in 1979.

USES

2-NP is used both as a solvent and as a chemical intermediate. Its largest reported single use of is as a solvent in paints (vinyl, epoxy, nitrocellulose, and chlorinated rubber) in concentrations ranging from 5% to 25%. It contributes such desirable properties as improved drying time, blush retardation, and greater wetting ability (International Minerals and Chemicals Corp., 1980; National Institute for Occupational Safety and Health, 1977). In 1977, 2-NP was reportedly used in the formulation of most of automotive paints (National Institute for Occupational Safety and Health, 1977). It is also used as a solvent in the production of vinyl resin-based clear varnishes for coatings on metal sheets produced for the manufacture of cans in the food industry, especially for beer cans, and in high-quality printing inks and in paint-removers (National

Institute for Occupational Safety and Health, 1977).

The compounds is used as an intermediate in the manufacture of a variety of chemicals, many of which are produced by International Minerals and Chemicals at its main plant in Sterlington, La., and in two other plants--at Terre Haute, Ind. and in Cologne, Federal Republic of Germany. One of the important derivatives is 2-nitro-2-methyl-1-propanol, which is used as an adhesive additive in the tire industry and in plastic and wood glues. It also functions as a bactericide and fungicide in cutting oils under the trade name Bioban P--1487 (Chemical Solvents Corporation, 1974; Anonymous, 1976.

2-NP is also used as a chemical intermediate in the production of 1-chloro-2-nitropropane. This chemical is registered with the U.S. Environmental Protection Agency (1974) for use as a soil fumigant for fruits and cotton crops.

EXPOSURE

No information is available on 2-NP released during production. One source (Science Applications, Inc., 1980) reports that Louisiana State files on air emissions indicate that "no 2-nitropropane emissions result from its production." This seems unlikely given the volatility of the compound; nevertheless, because production is confined to a single site, where the population is less than 5,000, emissions from production would be localized.

The most widespread source of exposure is the 2-NP used as a solvent. It can be assumed that all of this material is released into the atmosphere.

Exposures to higher concentrations of 2-NP should occur in industrial and commercial settings. A National Occupational Hazard Survey conducted by the National Institute for Occupational Safety and Health detected worker exposure to 2-NP in 217 different industries. These included industrial construction and maintenance, printing (rotogravure and flexographic inks), highway maintenance (traffic markings), shipbuilding and maintenance (marine coatings), furniture, food packaging, and plastic products. Overall, National Institute for Occupational Safety and Health, (1977) estimated that 100,000 workers have the potential for exposure to 2-NP.

The Food and Drug Administration (FDA) has allowed the use of 2-NP in the formulation of adhesives for articles intended for use in packaging, transporting, or holding food (21 CFR 175). In December 1978, the FDA proposed to delete provisions for such uses of 2-NP, having concluded that the substance is carcinogenic in animals. In January 1979, the FDA extended the comment period for the proposed ruling and, as of April 1980, no final ruling has been issued.

Exposure to 2-NP in the workplace is regulated by the Occupational Safety and Health Administration (OSHA). The time-weighted average exposure over 8 hours is not to exceed 25 ppm of 2-NP in air. However, as a result of carcinogenicity tests in animals, National Institute for Occupational Safety and Health (1977) recommended that 2-NP in the workplace be considered as a carcinogene in humans. OSHA has issued a Health Hazard Alert on 2-NP, which may serve to reduce the extent of industrial exposure (Occupational Safety and Health Administration, 1980).

ANALYTIC METHODS

Meyer and Locher (1975) were the first to recognize the characteristic reaction of secondary nitro compounds with nitrous acid to form blue pseudonitroles. Treon and Dutra (1952) used the reaction to determine airborne 2-NP that has been trapped in isopropyl alcohol. The absorbance at 277.5 nm was, however, not satisfactory for low concentrations. Jones (1963) extended the approach by trapping the 2-NP in concentrated sulfuric acid. When heated, the sulfuric acid decomposes 2-NP into nitrous acid, which is detected by its deep red-blue color reaction with resorcinol, usually measured at 560 nm . The technique was extensively evaluated by Jones (1963). The only compounds known to interfere were other substituted secondary nitro compounds. Primary nitroparaffins decompose to hydroxylamine under the conditions of the test and do not interfere with the determination of 2-NP. The method was sensitive to 3-5 μg of 2-NP.

Glaser (1978) developed a gas chromatographic (GC) method for 2-NP, sensitive at the 3-36 mg/m^3 level, utilizing a 3 liter air sample. A known amount of air was drawn through a sorbent tube containing 100 mg of Chromosorb 106, 60-80 mesh. After collection, the Chromosorb 106 was transferred to a stoppered container and desorbed with ethyl acetate. An aliquot of the ethyl acetate was analyzed by gas chromatography using a flame ionization detector. The claimed sensitivity of the method was 3 mg/m^3 at standard temperature and pressure, or 9 μg of 2-NP. Although easy to use, the method has two disadvantages. First, the absorbent is easily overloaded, and backup cartridges are required in order to check for overload. Second, the selectivity is poor when using a flame ionization detector. Presumably, this could be improved by utilizing a more specific GC detector such as a nitrogen-specific alkali bead flame ionization or a Hall detector, or a nitro-nitroso-specific system such as the thermal energy analyzer (TEA).

HEALTH EFFECTS

Metabolism

Some 2-NP is expired unchanged and some is converted to acetone and nitrite or nitrate by both animals and plants. Dequidt *et al.* (1973) reported finding considerable levels of nitrite in the heart, lung, kidney, spleen, and liver of rats given acute lethal or repeated nonlethal doses of 2-NP by intraperitoneal injection. The potential for 2-NP or the nitrite liberated from it to form *N*-nitroso compounds *in vivo* urgently needs attention.

Methemoglobinemia, presumably due to the interaction of nitrite and hemoglobin, was observed in the studies of acute exposure, but to a much lesser extent in the chronic exposure studies.

Acute Toxicity in Animals

Treon and Dutra (1952) reported on the inhalation toxicity of 2-NP in four species--rats, cats, rabbits, and guinea pigs. Two parameters were assessed: the highest atmospheric concentration the animals could tolerate without noteworthy immediate effects or after-effects and the lowest concentrations to induce mortality in a similar standard time exposure. These values are summarized in Table 6-1. The variation in toxicity between cats and guinea pigs is large--approximately sevenfold.

Table 6-1
Toxicity of 2-NP in Four Species after Exposure of 4.5 Hours[a]

Species	Highest "Nontoxic" Level (ppm)	Lowest Fatal Level (ppm)
Cat	328	714
Rat	714	1,510
Rabbit	1,400	2,380
Guinea pig	2,380	4,620[b]

---

[a] From Treon and Dutra, 1952, with permission.
[b] 3.5 hours exposure.

Rats, guinea pigs, and rabbits that inhaled air containing 2-NP at 325 ppm for 7 hours on 130 occasions (five per week) all survived. Similarly, the same species and one monkey survived 130 7-hour exposures to air containing 2-NP at 83 ppm spread over 190 days. Sufficient concentrations of 2-NP induced dyspnea, cyanosis, prostration and convulsions, and, finally, coma and death. There were also lacrimation, gastric regurgitation, and salivation. Widespread pathologic changes resulted from exposure to 2-NP at 2,350 ppm. Endothelial cells appeared to be most affected.

Machle *et al*. (1940) reported that an oral 2-NP dose of 0.25-0.50 g/kg was lethal to rabbits.

Chronic Toxicity in Animals

The first study reported was performed in rats and rabbits (Huntingdon Research Centre, 1977). Inhalation exposure of 50 male Sprague-Dawley rats to 2-NP at 400 ppm for 7 hours/day led to the death of 10 of the rats by the end of the second day and of 20 by the end of the third day. This group of rats was replaced by another, younger group, which was given 2-NP at 207 ppm. The replacement rats were considerably younger (weanling versus young adult) than those used in the rest of the experiment.

Groups of 50 rats and 15 rabbits were exposed by inhalation to 207, 27, or 0 ppm of 2-NP for 7 hours/day, 5 days/week. Ten rats and three rabbits were killed after 2 and 10 days and after 1, 3, and 6 months. All 10 high-dose rats killed after 6 months had liver tumors (hepatocellular adenoma or hepatocellular carcinoma); none of the other treated or untreated rats or rabbits showed this effect. Rats exposed for 3 months demonstrated hepatocellular hypertrophy, hyperplasia, and necrosis.

This experiment was criticized in Current Intelligence Bulletin #17 (National Institute for Occupational Safety and Health, 1977) which stated that the tumor-bearing animals started treatment at an earlier age than did either the low-exposure or control groups. This factor is probably of marginal importance to the assessment of the significance of the result. The induction of liver tumors in rats 6 months after the start of treatment indicates that 2-NP is a potent carcinogen in rats. Confirmation of this finding, using longer exposure times and lower levels of exposure, is clearly needed if meaningful risk assessments are to be formulated. Additional experiments are needed, for example, to exclude the possibility that there are metabolic or other thresholds with 2-NP that might lead to different tumor rates at high (as compared to low) exposures (Gehring and Blau, 1977).

International Minerals and Chemicals Corporation commissioned the Albany Medical College, N.Y. (Alamagordo Division) to perform further studies. Male and female Sprague-Dawley rats were exposed to 2-NP at a level of 200 ppm, 7 hours/day for 5 days/week. Animals were killed after 10 days and 1, 3, and 6 months. Ten animals treated for 3 months were removed from treatment and held for another 3 months in an atmosphere free of the test compound. Animals exposed for 6 months showed a variety of nonneoplastic liver changes; these included fatty metamorphosis and hepatocellular hypertrophy. Rats exposed for 3 months and allowed to recover showed evidence of regeneration.

In another study, male and female Sprague-Dawley rats were exposed to an atmospheric concentration of 2-NP at 100 ppm for 7 hours/day, 5 days/week for 18 months. Liver nodules were observed in 22 of 23 males and in 4 of 30 females, as compared to an incidence of 1 of 63 and 2 of 67, respectively, in unexposed animals. The histological diagnosis of these lesions does not seem to be available (International Minerals and Chemical Corporation, 1979b).

In an ongoing study by the same group, male and female Sprague-Dawley rats are being exposed by inhalation to 2-NP at 25, 100 and 200 ppm for 7 hours/day, 5 days/week. After 12 months, no benign or malignant hepatocellular neoplasms were reported after gross or microscopic examination. Completion and release for peer review of the Albany Medical College's findings on the toxicity and carcinogenicity of 2-NP are urgently needed. A Health Hazard Alert by the Occupational Safety and Health Administration (1980) gives further information on this study and indicates that 2-nitropropane at 200 and 100 ppm induces liver cancer in these rats, whereas 25 ppm failed to do so by 22 months of exposure.

Acute Toxicity in Humans

Skinner (1947) described the toxic effects in humans exposed to 2-NP in paints. He noted that five or six workers were exposed to 2-NP in concentrations between 20 and 45 ppm. Those least exposed experienced symptoms of severe headache. Those exposed to the higher concentrations experienced anorexia, nausea, vomiting, and diarrhea. When methyl ethyl ketone was substituted for the 2-NP, the above symptoms were no longer reported. Several workers intermittently exposed to 10-30 ppm were not adversely affected. These latter exposures did not exceed 4 hours/day or more than 3 days/week.

Hine et al. (1978) detailed the clinical symptoms and macroscopic and microscopic findings in four fatal cases believed to have resulted from overexposure to 2-NP. Two of four men were using vinyl paints; one was using a coal tar surface-coating; the fourth was using a polyester-based resin. Death occurred 6 to 10 days after exposure; post mortem findings indicated liver necrosis in three cases and fatty degeneration of the liver in the fourth. A fifth, nonfatal case involved a printer who spilled 2-NP, which he had used as a solvent, on the floor. In no case was there any information on the level of exposure to 2-NP or exposure to other solvents.

Gaultier et al. (1964) described one fatality and one recovery from exposure to high levels of 2-NP. The men had been painting the inside of a tank. Both suffered liver damage.

Lower levels of 2-NP exposure (20-45 ppm) led to nausea, vomiting, and diarrhea in workers at one plant (International Minerals and Chemicals Corporation, 1979b). In addition, Williams *et al.* (1974) reported an increased incidence of toxic hepatitis among construction workers applying epoxy resins to the walls of a nuclear power plant; however, the hepatotoxicity could have been due to exposure to methylenedianiline, a well-documented potent hepatotoxin, rather than to exposure to 2-NP.

Exposure of humans to toxic levels of 2-NP does not appear to be frequent or particularly well documented. Data for each of the examples discussed in this section are incomplete, leaving open the question of whether the outcome was dependent on other environmental contaminants, acting either alone or in conjunction with 2-NP.

Mutagenicity

Only one published and three unpublished mutagenicity studies of 2-NP exist. It is a mutagen for *Salmonella* in all studies, but failed to induce micronuclei in polychromatic erythrocytes in mice.

Bacteria. Hite and Skeggs (1979) showed that 2-NP induced mutations in *Salmonella typhimurium* strains TA92, TA98, TA100, and TA1537 in the standard plate test, without metabolic activation, and in the presence of Aroclor- and phenobarbital-induced rat liver S-9. The responses attained with S-9 were higher than the corresponding responses without S-9. No differences were observed between Aroclor and phenobarbital induction. The presence of S-9 on the plate appeared to decrease the toxicity induced by 2-NP. The source and purity of the 2-NP used were not described. 1-Nitropropane was not mutagenic in this study.

Technical-grade 2-NP (Metheson/Coleman and Bell 2-nitropropane, 93.1%; 1-nitropropane, 6.7%; acetone, 0.2%) was tested in two laboratories (Stanford Research Institute International and Case Western Reserve University) through the Environmental Mutagenesis Test Development Program of the National Institute for Environmental Health Sciences. The test system used was a preincubation modification of the Salmonella/microsome test, using Aroclor-induced rat and Syrian hamster liver S-9 and Salmonella strains TA98, TA100, TA1535, and TA1537. 2-NP was mutagenic for TA98 and TA100 in one laboratory; TA98, TA100, and TA1535 demonstrated mutagenicity in the second. Again, mutagenicity was observed both with and without S-9, but the response without S-9 was weaker. Subsequent studies with nitroreductase-deficient Salmonella have shown that bacterial reduction of the nitro group does not appear to be responsible for 2-NP's mutagenic activity (Mortelmans and Speck, personal communication, 1980).

2-NP of undefined purity was tested for Mobil Chemical Company by Litton Bionetics, Inc. (1977). The Salmonella plate test with

TA98, TA100, TA1535, TA1537, and TA1538 was used. Also, the yeast *Saccharomyces cerevisiae* D4, which is used to detect mitotic gene conversion, was used in a plate-test protocol identical to that used for *Salmonella*. A positive mutagenic response was reported only for TA98 at levels of 10 and 20 μl (equivalent to approximately 10 and 20 mg/plate) with Aroclor-induced rat liver S-9. The other *Salmonella* strains and the yeast were tested at a concentration of 5 μl/plate. It is difficult to interpret the negative response observed with yeast because the protocol employed was a departure from published techniques and had not been demonstrated to be effective with standard mutagens.

A parallel test series with 1-nitropropane yielded negative results.

Mammals. For the micronucleus test in CD-1 mice, 2-NP was administered orally, twice daily, at 0.1, 0.2, and 0.3 mg/kg/day. (The 14-day oral LD_{50} was 0.40 mg/kg). Bone marrow erythrocytes (polychromatic and normochromatic) were examined for micronuclei; significant effects were reported (Hite and Skeggs, 1979).

Teratogenicity

There were no data from which to evaluate the potential teratogenicity on reproductive toxicity of 2-NP.

Mortality Study in Humans

International Minerals and Chemicals Corporation (IMC), the sole commercial U.S. manufacturer of 2-NP, started work in Louisiana in 1946. 2-NP production was introduced there in 1955. IMC (1979 a,b) reported on the mortality experience of all 1,815 persons who had, at one time or another, been employed at the plant between 1946 and 1977. A record file was established to continue surveillance of this population.

By June 30, 1977, 180 of these employees had died. Mortality was compared, by cause, for different subpopulations of the work force. Overall, the 1,066 white male employees showed a standardized mortality ratio (SMR) of 85% as compared to an equivalent age-, race-, and sex-matched segment of the total U.S. population. Such a value is usual for occupational work groups which, through normal hiring practices, exclude the physically unfit. Accidents (motor vehicle, for example) had occurred more frequently than expected. There was no evidence of an increased incidence of liver cancer in this group. There were two lymphatic tumors; only 0.8 were expected, but the finding was not statistically significant.

Among the 208 black males, the SMR was 67%. There was no statistically significant increase in mortality from any cause, although lymphatic tumors again were at increased incidence (two observed vs 0.2 expected).

The total study population was then divided into people who had been (1) directly, (2) indirectly, and (3) not exposed to 2-NP. The increased incidence of lymphatic neoplasia, in both black and white males, was confined to those not exposed to 2-NP. Analysis failed to show that 2-NP exposure was associated with any particular cause of death. Similarly, there was no evidence of an increased mortality rate when the duration of exposure to 2-NP was considered.

It must be concluded that employment at this large-scale, 2-NP manufacturing plant did not increase cancer mortality in the work population; however, maximum exposure to 2-NP lasted only 22 years and some occupational tumors have a longer latency. Second, levels of 2-NP in the environment were monitored; with few exceptions (such as drum fillers), the present occupational standard for 2-NP was not exceeded. Third, although the total population exposed for 15 or more years was not reported, it appears to be a small fraction of the total.

Thus, this survey, so far, is reassuring about the potential human hazard of manufacturing 2-NP under adequate environmental conditons. However, it is still too early to be certain that 2-NP does not lead to the induction of cancer in the population exposed to it. Thus, the company should be encouraged to continue environmental and epidemiologic surveillance for several more decades.

CONCLUSIONS

At high atmospheric concentrations, 2-NP is hepatocarcinogenic in rats. There is a latency of 6 months. Rats are the only experimental species tested adequately. Results of inhalation testing at 25 ppm have not yet been reported. The results of the chronic toxicity study performed by the Albany Medical College for the International Minerals and Chemical Corporation must be peer reviewed and published before that data can be used for any kind of risk assessment.

2-NP is mutagenic in *Salmonella*, producing both frame-shift and base-pair substitution mutations, both with and without added liver S-9. There is no evidence on 2-NP's ability to induce point mutations in mammalian cells. The negative results of the micronucleus test in mice are not sufficient to label 2-NP as nonclastogenic; *in vitro* and *in vivo* cytogenetic assays are needed. In addition, there is a need for more data on potential carcinogenicity, other toxicity, teratogenicity, and other reproductive effects.

The one epidemiologic study reported failed to demonstrate that 2-NP was carcinogenic in humans, but additional evaluation over a longer period is required before it can be accepted that 2-NP is not carcinogenic in humans under the conditions at the Sterlington plant. The volatility of 2-NP causes it to attain high concentrations in the work environment. Because of its acute toxicity at high concentrations and its reported carcinogenicity in rats, the use of 2-NP requires caution in the occupational and general environment. Moreover, the levels to which humans are currently exposed should be carefully monitored. The appropriateness of the presently permitted threshold limit value/time weighted average adapted by the American Conference of Governmental Industrial Hygienists (1980) is still debatable. However, the major hazard associated with 2-NP appears to be the consequences of its release into a confined, unventilated space from paints, resins, and similar coating materials in which it is used as a solvent.

REFERENCES

Production, Uses, Exposure

Anonymous. 1976. Grande Paroisse looks for growth in nitroparaffins market. Eur. Chem. News 29:23-24,30, August 6.

Chemical Solvents Corporation. 1974. Buyer's. Chemical Solvents Corporation, New York.

Code of Federal Regulations. 1980. Title 21, Part 175. Indirect food additives: Adhesive coatings and components. Office of the Federal Register, National Archives and Records Service, General Services Administration, Washington, D.C.

International Minerals and Chemicals Annual Reports. 1977, 1978, 1979. International Minerals and Chemicals Corp., Northbrook, Ill.

International Minerals and Chemicals Corp. 1980. Technical Bulletin. Des Plaines, Ill.

National Institute for Occupational Safety and Health. 1977. 2-Nitropropane. Current Intelligence Bulletin 17. U.S. Department of Health, Education, and Welfare, Office of Extramural Coordination and Special Projects, Rockville, Md. 6 pp.

Occupational Safety and Health Administration. 1980. Health Hazard Alert--2-Nitropropane (2-NP). DHHS(NIOSH) Publication No. 80-142. U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, National Institute for Occupatonal Safety and Health, Rockville, Md.

Science Applications, Inc. 1980. Human Exposure to Atmospheric Concentrations of Selected Chemicals, Draft Report No. EF-156R to U. S. Environmental Protection Agency, Science Appllcation, Inc., March 1980.

Stanford Research Institute International. 1979. 1979 Directory of Chemical Producers: Western Europe. 2 volumes. Stanford Research Institute International, Menlo Park, Calif. 2,100 pp.

U.S. Environmental Protection Agency. 1974. EPA Compendium of Registered Pesticides, Volumes I-V. U.S. Environmental Protection Agency, Washington, D.C.

Analytic Methods

Glaser, R.A. 1978. 2-Nitropropane. Pp. 272-1 - 272-9 in NIOSH Manual of Analytical Methods, Vol. 4, 2nd ed. D.G. Taylor, Manual Coordinator. DHEW(NIOSH) Publication No. 78-175. U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

Jones, L.R. 1963. The determination of 2-nitropropane in air. Am. Ind. Hyg. Assoc. J. 24:11-16.

Meyer, V., and J. Locher. 1975. Ueber die Einwirkung der Sauren auf nitrirte Fettkorper. Ber. Dtsch. Chem. Ges. 8:219-221.

Treon, J.F., and F.R. Dutra. 1952. Physiological response of experimental animals to the vapor of 2-nitropropane. Arch. Ind. Hyg. Occup. Med. 5:52-61.

Health Effects

American Conference of Governmental Industrial Hygienists. 1980. Threshold limit values for chemical substances in workroom air adopted by ACGIH for 1980, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio.

Dequidt, J., J.M. Haguenour, J. Potencier, and P. Vasseur. 1973. Etude toxicologique experimentale du 2-nitropropane. Arch. Mal. Pro. 34:59-63.

Gaultier, M., P.E. Fournier, P. Gervais, and J. Beusnel. 1964. Possibilities d'action d'un Centre Regional de Toxicologie en Medicine du Travail. Arch. Mal. Prof. Med. Trav. Secur. Soc. 25: 430-436.

Gehring, P.J., and G.E. Blau. 1977. Mechanisms of carcinogenesis: Dose response. J. Environ. Pathol. Toxicol. 1:163-179.

Hine, C.H., A. Pasi, and B.G. Stephens. 1978. Fatalities following exposure to 2-nitropropane. J. Occup. Med. 20:333-337.

Hite, M., and H. Skeggs. 1979. Mutagenic evaluation of nitroparaffins in the Salmonella typhimurium/mammalian-microsome test and the micronucleus test. Environ. Mutagenesis 1: 383-389.

Huntingdon Research Center. 1977. Final Report: Chronic Inhalation Exposure of Rats and Rabbits to Nitromethane and 2-Nitropropane. HEW/NIOSH Project No. 210-75-0039, H.R.C Project No. 758-209. Huntingdon Research Center, New York. 220 pp.

International Minerals and Chemical Corp. 1979a. 2 NP Mortality Epidemiology Study of the Sterlington, La. Employees 1-1-46 thru 6-30-77, by M.E. Miller and G.W. Temple. International Minerals & Chemical Corp., Mundelein, Ill. [44] pp.

International Minerals and Chemicals Corp. 1979b. Review on safety data on 2-nitropropane (2-NP). NP Division, International Minerals & Chemical Corp., Des Plaines, Ill. 23 pp.

Litton Bionetics Inc. 1977. Mutagenicity evaluation of MCTR-185-77. Final Report. 2-Nitropropane. Submitted to Mobil Chemical Company August, 1977. Litton Bionetics, Inc., Kensington, Md. [10] pp.

Machle, W., E.W. Scott, and J. Treon. 1940. The physiological response of animals to some simple mononitroparaffins and to certain derivatives of these compounds. J. Ind. Hyg. Toxicol. 22:315-332.

National Institute for Occupational Safety and Health. 1977. 2-Nitropropane. Current Intelligence Bulletin 17. U.S. Department of Health, Education, and Welfare, Office of Extramural Coordination and Special Projects, Rockville, Md. 6 pp.

Occupational Safety and Health Administration. 1980. Health Hazard Alert--2-Nitropropane (2-NP). DHHS(NIOSH) Publication No. 80-142. U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, National Institute for Occupatonal Safety and Health, Rockville, Md.

Skinner, J.B. 1947. The toxicity of 2-nitropropane. Ind. Med. Surg. 16:441-443.

Treon, J.F., and F.R. Dutra. 1952. Physiological response of experimental animals to the vapor of 2-nitropropane. Arch. Ind. Hyg. Occup. Med. 5: 52-61.

Williams, S. V., J.A. Bryan, J.F. Burk, and F.S. Wolf. 1974. Toxic hepatitis and methylenedianiline. New Engl. J. Med. 291:1256.